these experiments, was 0.035 *M*. Incubation of the reaction mixtures and determination of glucose released on hydrolysis were carried out as described above.

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Proton Magnetic Resonance Spectra of Tetracyclines

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The investigational tool of n.m.r. spectroscopy has been applied rather infrequently to the solutions of problems posed by the chemistry of tetracyclines. In several instances, however, this method has been used successfully to clarify certain structural or stereochemical features of some tetracycline molecules.[1-7] Since the published data are few and dispersed widely, it is the intention of this paper to make available the information obtained in these laboratories by n.m.r. spectroscopy on a variety of tetracycline derivatives. The usefulness of these data has already been demonstrated in that they permitted structural, stereochemical, and conformational assignments hitherto not possible and it is anticipated that they will be of value in future investigations.

Generally, n.m.r. spectra of tetracyclines are complex and difficult to obtain for lack of suitable solvents. Several derivatives, however, are more amenable to this method and served as a starting point for our investigations.

Since the complete stereochemistry of oxytetracycline (**3**) has been elucidated recently,[5] it is now possible also to determine the conformations of several oxytetracycline derivatives in solution, a matter of interest particularly to those concerned with reaction mechanisms and drug–enzyme interactions. It now appears that the principal conformation for these derivatives is similar to that shown in Figure 1 for the parent compound. This is different from that derived tentatively from X-ray studies,[8] but it fulfills the following conditions: planarity of the two β-diketone systems C-11–C-12 and C-1–C-3, *trans*-diaxial relationship of the protons attached to C-4 and C-4a, and a pseudo-equatorial–pseudo-axial relationship of the two protons attached to C-5 and C-5a (*vide infra*). The conformation shown can roughly be described as holding the molecule in two plains, intersecting with each other at an angle of about 110° along a line connecting C-5 and C-12a. However, there is a certain amount of puckering of rings A and B.

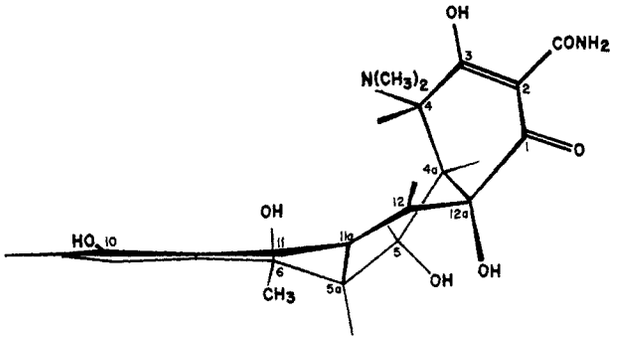
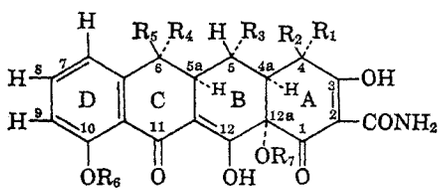
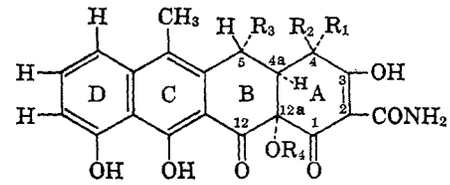


Figure 1.

The data shown in Table I were obtained in the solvents indicated, using tetramethylsilane as internal standard with a Varian A-60 instrument. When the chemical shift could not be determined unequivocally because of overlapping peaks, the range encompassing the signal under discussion is indicated. The coupling constants cited below were obtained directly from the spectra by simple first-order treatment.



	R1	R2	R3	R4	R5	R6	R7
1	$N(CH_3)_2$	H	H	CH_3	OH	H	H
2	H	$N(CH_3)_2$	H	CH_3	OH	H	H
3	$N(CH_3)_2$	H	OH	CH_3	OH	H	H
4	$N(CH_3)_2$	H	H	H	CH_3	H	H
5	$N(CH_3)_2$	H	OH	CH_3	H	H	H
6	$N(CH_3)_2$	H	OH	H	CH_3	H	H
7	$N(CH_3)_2$	H	H	CH_2		H	H
8	$N(CH_3)_2$	H	OH	CH_2		H	H
12	$N(CH_3)_2$	H	OH	CH_3	H	H	Ac
13	$N(CH_3)_2$	H	OAc	CH_3	OH	H	H
14	$N(CH_3)_2$	H	OAc	CH_3	OH	H	Ac
16	$N(CH_3)_2$	H	OH	CH_3	OH	Ac	Ac



	R1	R2	R3	R4
9	$N(CH_3)_2$	H	H	H
10	H	$N(CH_3)_2$	H	H
11	$N(CH_3)_2$	H	OH	H
15	$N(CH_3)_2$	H	OAc	Ac

The proton attached to C-4 gives a signal between 3.6 and 4 p.p.m. in pyridine solution, while in chloroform this peak shifts about 0.3 p.p.m. upfield. Protonation of the amino nitrogen causes this signal to shift downfield and in trifluoroacetic acid it generally falls

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TABLE I
PROTON RESONANCE DATA FOR TETRACYCLINES
(in parts per million)

Compound	Solvent	C-4 H	C-4a H	C-5 H	C-5a H	C-6 H	$N(CH_3)_2$	C-6 CH_3	C-6 $=CH_2$	$-OCO_3$
Tetracycline (1)	C_5H_5N	3.6	2.2–3.2	2.2–3.2	2.2–3.2		2.6	1.8		
4-Epitetracycline (2)	C_5H_5N	4.6	2.2–3.2	2.2–3.2	2.2–3.2		2.7	1.8		
Oxytetracycline, base (3)	C_5H_5N	4.1	3.1	5.3	3.4		2.7	2.2		
HCl	DMSO	3.8	2.9	4.8	2.9		2.9	1.7		
β-6-Deoxytetracycline[a] (4)	CF_3COOH	4.1					3.3	1.0		
α-6-Deoxyoxytetracycline[a] (5)	C_5H_5N	3.9	2.8–3.1	4.5	2.8–3.1	2.8–3.1	2.7	1.7		
	CF_3COOH	4.1	2.7–3.4	4.6	2.7–3.4	2.7–3.4	3.3	1.7		
β-6-Deoxyoxytetracycline[a] hydrochloride (6)	C_5H_5N	3.9–4.3	3.0–4.3	5.3	3.0–4.3	3.0–4.3	3.3	1.0		
	CF_3COOH	4.1	3.1–3.6	4.8	3.1–3.6	3.1–3.6	3.3	1.1		
6-Methylenetetracycline[b] (7)	CF_3COOH						3.3		5.2, 5.7	
	$CDCl_3$						2.5		5.1, 5.5	
6-Methyleneoxytetracycline[b] (8)	CF_3COOH			4.8			3.3		5.5, 5.7	
Anhydrotetracycline (9)	C_5H_5N	2.8–3.8	2.8–3.8	2.8–3.8			2.6	2.4		
	CF_3COOH	4.2	3.1–3.7	3.1–3.7			3.3	2.4		
4-Epianhydrotetracycline (10)	CF_3COOH	5.2	3.0–3.9	3.0–3.9			3.5	2.3		
Anhydrooxytetracycline (11)	CF_3COOH	4.4	3.8	5.8			3.3	2.7		
12a-Acetyloxytetracycline[c] (12)	C_5H_5N	4.0	4.2	5.5	3.4		2.6	2.2		2.3
5-Acetyloxytetracycline[c] (13)		4.0	3.1	(6.7)	3.4		2.7	1.9		2.2
5,12a-Diacetyloxytetracycline[c] (14)	C_5H_5N	4.0	4.1	6.7	3.2		2.7	1.9		2.1, 2.3
	C_5H_5N									
	$C_4H_8O_2$			6.2			2.5	1.7		2.0, 2.1
5,12a-Diacetylanhydrooxytetracycline[c] (15)	C_5H_5N	3.8	4.5	>7.0			2.7	2.4		2.2, 2.2
	CF_3COOH	4.5	4.7	6.8			3.4	2.6		2.4, 2.4
	$CDCl_3$	3.4	4.1	7.0			2.6	2.4		2.1, 2.2
10,12a-Diacetyloxytetracycline[c] (16)	C_5H_5N	3.8	4.1	5.5	3.4		2.5	2.2		2.3, 2.3

[a] Reference 2. [b] Reference 3. [c] R. K. Blackwood, P. N. Gordon, M. Schach von Wittenau, and C. R. Stephens, paper in preparation.

in the range of 4–4.5 p.p.m. Presence of a hydroxyl group at C-5 appears to be of little influence (**4** *vs.* **6**) upon the chemical shift of the C-4 proton.

The magnitude of the coupling constant between the C-4 and C-4a protons was not apparent from all spectra. Especially when the amine nitrogen is protenated the C-4 proton signal is a multiplet in analogy to that of the N-methyl groups which generally appear as two doublets in acidic solvents. For oxytetracycline (**3**) and its 6-deoxy derivative **5**, however, the apparent coupling constant is 9 c.p.s., and, for the oxytetracycline derivatives **12–16**, the corresponding value is 11–13 c.p.s. For these compounds the relationship of the C-4 and C-4a protons therefore must be *trans* diaxial.

The same conclusion may not be drawn from the spectrum of tetracycline (**1**). Here the apparent coupling constant is 4.5 c.p.s. Although this first-order treatment may be insufficient, it is interesting to note that the conformation determined by X-ray studies, *i.e.*, an eclipsed conformation about the C-4–C-4a bond,[8] accommodates this value. This difference in conformations between tetracycline (**1**) and oxytetracycline (**3**) could be explained by the interactions in the latter molecule (**3**) of the C-5 hydroxyl group with substituents at C-6, thus forcing the C-5 hydroxyl group into the pseudo-axial position and the A and B rings into conformations different from those in tetracycline (**1**).

Epimerization at C-4 causes a chemical shift downfield of about 1 p.p.m. (**1** *vs.* **2** and **9** *vs.* **10**). An attractive explanation for this phenomenon is the increased basicity of the dimethylamino group[6] and the change of spacial relationship of the C-4 proton in relation to the planar β-diketone system C-1–C-3 from a quasi-perpendicular to a more coplanar one. The apparent coupling constant for **2** is 5 c.p.s. and is thus consistent with the suggested conformation.

The chemical shift of the C-4a proton is of the order of magnitude of 3 p.p.m. It is of interest that acetylation of the C-12a hydroxyl group causes a large shift downfield of about 1 p.p.m. while acetylation of the C-5 hydroxyl is of minor influence. Probably, electrostatic repulsion of the carbonyl of the C-12a acetoxy group by the C-1 and C-12 carbonyls forces the former into close proximity to the C-4a hydrogen, thus causing this large shift. In the 12a-acylated molecules the chemical shifts of the C-4 and C-4a protons are similar, and for the diformyl analog of **14** they are identical, appearing as a sharp singlet in pyridine solution.

The apparent coupling constant between the C-4a and C-5 protons for those molecules where it could be determined (oxytetracycline derivatives **12–16**) is always very small, about 0–2 c.p.s., implying an approximate right-angle relationship for these two protons. Solvents do not appear to influence the conformation much as demonstrated by **15**, where the respective

coupling constants $J_{4,4a}$ and $J_{4a,5}$ are the same for the three solvents studied.

The signal for the C-5 proton in oxytetracycline appears rather far downfield at about 5 p.p.m. Acylation of the C-5 hydroxyl causes a large shift of over 1 p.p.m. downfield. Paramagnetic shifts are also caused, predictably, by aromatization of the C ring.

The splitting pattern is not always clear. Sometimes a multiplet with a half-width of 5–11 c.p.s. is seen; sometimes a doublet can be discerned (3–4 c.p.s.) that is also found in the signals for C-4a or C-5a protons, depending upon the particular molecule under study. In no case does the splitting pattern imply a *trans*-diaxial relationship of the C-5 proton to its adjacent protons; rather a pseudo-equatorial conformation is suggested.

The signal for the C-5a proton appears generally at about 3 p.p.m., unless it becomes allylic, in which case a paramagnetic shift is observed. The coupling constant with the C-5 proton is never greater than 4 c.p.s., sometimes much less, when only a somewhat broadened singlet is seen.

The β-6-deoxy derivatives **4** and **6** show the signal for the C-6 methyl group farther upfield than the α analog **5**. This finding is consistent with the assigned stereochemistry[2] since it implies a pseudo-axial position for the C-methyl group in the β isomer and a pseudo-equatorial position for the α epimer. In corraboration, the C-methyl signal for **4** and **6** appears as a doublet (J = 6–8 c.p.s.), while the corresponding peak for **5** is a broadened singlet.

The signals for the aromatic protons show the expected chemical shift. Sometimes the splitting pattern is simple; *i.e.*, for **3** (DMSO) a triplet appears at 7.6 and two doublets at 7 and 7.2 p.p.m., respectively. The signal for the C-8 proton appears at 6.8 p.p.m. when C-7 is substituted by chlorine and C-9 by a *t*-butyl group[4] ($CHCl_3$). Without the *t*-butyl group but with a C-7 chlorine,[4] two doublets appear at 7.0 and 7.6 p.p.m. ($CHCl_3$). Interesting data on the relation of chemical shift and degree of ionization have been published recently.[6]

The signals for the easily exchangeable protons,[9] comprising the O–H and N–H, can be found between 4 and 16 p.p.m. and are greatly solvent dependent. In several instances these labile protons were exchanged for deuterium ions to make definite assignments of peaks possible.

Acknowledgment.—We are indebted to Mr. R. Hickey for the spectral measurements.

(9) A-Ring models have been studied: *cf.* G. O. Dudek and G. P. Volpp, *J. Org. Chem.*, **38**, 50 (1965).

Oxidations with Manganese Dioxide

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Manganese dioxide has proved a valuable oxidizing agent for certain functional groups. In addition to α,β-unsaturated primary and secondary alcohols, which are converted to the corresponding aldehydes and ketones, many other compounds are oxidized smoothly by this reagent.[1]

More recently, manganese dioxide has been used in effecting a variety of transformations: methylpyridinemethanols to methylpyridinecarboxaldehydes[2]; 2-indolemethanol to 2-indolecarboxaldehyde[3]; diarylmethanes to tetraarylethanes or diaryl ketones[4]; phenylhydrazides to carboxylic acids[5,6]; primary aromatic amines and hydrazobenzenes to azobenzenes[7,8]; N-benzylanilines to benzalanilines[8]; 1,2,3,4-tetrahydroquinoline, 2,3-dihydroindole, and acridane to quinoline, indole, and acridine, respectively[8]; simple and tricyclic indolines to indoles[9]; and quinaldine, lepidine, α- and γ-picoline, and 1-methylisoquinoline to the corresponding carboxylic acids.[10] It has also been reported that primary and secondary saturated alcohols are oxidized to the carbonyl compounds in good yields when sufficient quantities of the oxidant and pure solvents are used.[11]

In this laboratory we have used manganese dioxide to oxidize in good yields pyridinemethanols to the corresponding aldehydes, mercaptans to disulfides,[12] aliphatic α-hydroxy ketones to 1,2-diketones, and N-phenylhydroxylamine to nitrosobenzene. The general experimental procedure is as follows. A suspension of manganese dioxide in a solution of the substance to be oxidized was stirred vigorously for 5–6 hr and the oxide was removed by filtration and washed with ether. The filtrate and washings were concentrated under reduced pressure, and the product was isolated by distillation or recrystallization. The results are summarized in Table I.

Some of the oxidations described in this paper may be effected in comparable yields by other reagents: lead tetraacetate (pyridinemethanols to pyridinecarboxaldehydes,[13] thiols to disulfides[14]); selenium dioxide (methylpyridines[15] and pyridinemethanols[16] to pyridinecarboxaldehydes); concentrated nitric acid [imidazole-4- (or 5-) methanol to the aldehyde[17]]; dimethyl sulfoxide (thiols to disulfides[18]); and cupric acetate, ferric chloride,[19] or bismuth oxide[20] (acyloins to diketones). The present method deserves consideration as a synthetic route by virtue of its simplicity, selectivity, and ease of product isolation.

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