Chemistry of Tetracyclines

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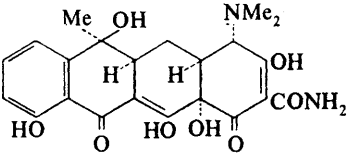
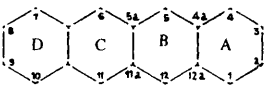
1 Introduction

The tetracyclines are a group of compounds which has afforded the medical profession a number of powerful antibiotics active against a wide range[1,2] of human and animal pathogens. The compounds are closely related to the parent substance, tetracycline(I), and they are obtained as metabolic products of various forms of *Streptomyces*, or produced by chemical modification of the natural metabolites. Nomenclature is based on the numbering shown in (*A*).



(I) Tetracycline



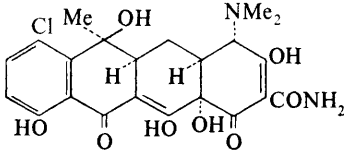
Because of their complex structure and great therapeutic value [*e.g.*, the number of prescriptions for antibiotics dispensed in England during 1966 is estimated[3] to be (millions): penicillins, 14·6; tetracyclines, 12·6; others, 3·3.] the tetracyclines have been the object of numerous studies.[4] Their mode of action,[5] biogenesis,[6–10] and chemical properties have been examined extensively, and the prospect of synthesising a natural member of the group has received a great deal of attention.[5] This Review summarises the results of structural work and examines the published reactions. Such a study will show the circumstances

[1] (*a*) L. M. Pruess and C. H. Demos, in 'Encyclopedia of Chemical Technology', ed. R. E. Kirk and D. F. Othmer, Interscience, New York, 1954, vol. 13, p. 785; (*b*) P. P. Regna, *ibid.*, pp. 800, 808.
[2] H. K. Spitzy, *Antibiotica et Chemotherapia*, 1962, **10**, 193.
[3] Annual Report of The Ministry of Health for the Year 1966, Cmnd. 3326, H.M.S.O., London, 1967, p. 95.
[4] H. Muxfeldt and R. Bangert, *Fortschr. Chem.-org. Naturstoffe*, 1963, **21**, 80.
[5] G. C. Barrett, *J. Pharm. Sci.*, 1963, **52**, 309.
[6] J. R. D. McCormick, in 'Biogenesis of Antibiotic Substances', ed. Z. Vanek and Z. Hostalek, Academic Press, New York, 1965, p. 73.
[7] J. H. Martin, L. A. Mitscher, P. A. Miller, P. Shu, and N. Bohonos, Antimicrobial Agents and Chemotherapy, *Amer. Soc. Microbiol.*, Ann Arbor, Michigan, 1966, p. 563.
[8] P. A. Miller, A Saturnelli, J. H. Martin, L. A. Mitscher, and N. Bohonos, *Biochem. Biophys. Res. Comm.*, 1964, **16**, 285.
[9] J. R. D. McCormick, U. H. Joachim, E. R. Jensen, S. Johnson, and N. O. Sjolander, *J. Amer. Chem. Soc.*, 1965, **87**, 1793.
[10] P. A. Miller, J. H. Hash, M. Lincks, and N. Bohonos, *Biochem. Biophys. Res. Comm.*, 1965, **18**, 325.

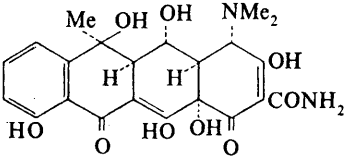
in which the tetracyclines are unstable and will illustrate the extent to which their sensitivity can be reduced by temporary modification. There will also be an opportunity to screen the reactions for possible use in total or partial synthetic work.

2 Structural Results

The fundamental discoveries in the tetracycline field were made over a period of six years.[11] The first member of the group, 7-chlorotetracycline (2), was



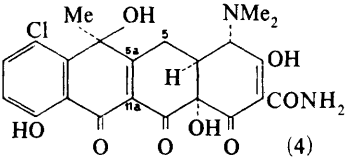
(2) Aureomycin



(3) Terramycin

isolated[12,13] in 1947 and, being effective against a wider range of pathogenic micro-organisms than any agent in use up to that time, it soon became important in medical practice, in which it was known by the trade name, aureomycin. A few years later 5-oxytetracycline, or terramycin (3), was discovered[14,15] and it, too, acquired great importance. Finally, in 1953, hydrogenolysis of aureomycin was reported to give[16,17] tetracycline (1). This compound, obtained subsequently also by fermentation,[18] represents the molecular structure common to the other antibiotics and formally it is the parent for a number of compounds isolated later. (Not all the tetracyclines nor, indeed, all their laboratory derivatives, are useful as antibiotics; tetracycline therapy involves mainly the first three members and the more recent discovery, 7-chloro-6-demethyltetracycline.) These include the 7-bromo-,[19,20] 6-dimethyl-,[21,22] and 7-chloro-6-demethyl[21,22] derivatives, a

[11] J. H. Boothe, Antimicrobial Agents and Chemotherapy, *Amer. Soc. Microbiol*, Ann Arbor, Michigan, 1962, p. 213.
[12] B. M. Duggar, *Ann. New York Acad. Sci.*, 1948, **51**, 177.
[13] R. W. Broschard, A. C. Dornbush, S. Gordon, B. L. Hutchings, A. R. Kohler, G. Krupka, S. Kushner, D. V. Lefemine, and C. Pidacks, *Science*, 1949, **109**, 199.
[14] A. C. Finlay, G. L. Hobby, S. Y. P'an, P. P. Regna, J. B. Routien, D. B. Seeley, G. M. Shull, B. A. Sobin, I. A. Solomons, J. W. Vinson, and J. H. Kane, *Science*, 1950, **111**, 85.
[15] P. P. Regna, I. A. Solomons, K. Murai, A. E. Timreck, K. J. Brunings, and W. A. Lazier, *J. Amer. Chem. Soc.*, 1951, **73**, 4211.
[16] L. H. Conover, W. T. Moreland, A. R. English, C. R. Stephens, and F. J. Pilgrim, *J. Amer. Chem. Soc.*, 1953, **75**, 4622.
[17] J. H. Boothe, J. Morton, jun., J. P. Petisi, R. G. Wilkinson, and J. H. Williams, *J. Amer. Chem. Soc.*, 1953, **75**, 4621.
[18] P. P. Minieri, M. C. Firman, A. G. Mistretta, A. Abbey, C. E. Brickler, N. E. Rigler, and H. Sokol, 'Antibiotics Annual 1953—1954', Medical Encyclopedia, Inc., New York, 1953, p. 81.
[19] A. P. Doerschuk, J. R. D. McCormick, J. J. Goodman, S. A. Szumski, J. A. Growich, P. A. Miller, B. A. Bitler, E. R. Jensen, M. Matrishin, M. A. Petty, and A. S. Phelps, *J. Amer. Chem. Soc.*, 1959, **81**, 3069.
[20] P. Sensi, G. A. De Ferrari, G. G. Gallo, and G. Rolland, *Il Farmaco Edizione Scientifica*, 1955, **10**, 337.
[21] J. R. D. McCormick, N. O. Sjolander, U. Hirsch, E. R. Jensen, and A. P. Doerschuk, *J. Amer. Chem. Soc.*, 1957, **79**, 4561.
[22] J. S. Webb, R. W. Broschard, D. B. Cosulich, W. J. Stein, and C. F. Wolf, *J. Amer. Chem. Soc.*, 1957, **79**, 4563.



(4)

number of 2-acetyl-2-decarboxyamidotetracyclines,[23,24] and several dehydro-compounds[25] of which 7-chlorodehydrotetracycline (4) has been described[26–28] in most detail. The position of the extra double bond in the last substance has not been settled; probably the different tautomers[27] have the double bond at positions 5,5*a* or 5*a*,11*a*.

The structures of terramycin[29] and aureomycin[30,31] were established by chemical experiments. Then, with the reactions and spectral attributes of the tetracyclines extensively defined, assignment of structures to other members of the group was greatly simplified and followed closely on their isolation. The structure of tetracycline was apparent from its simple relationship to aureomycin (from which it can be obtained by hydrogenolysis).[16,17]

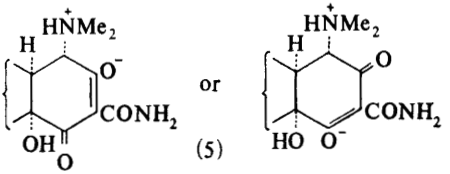
An *X*-ray analysis of aureomycin hydrochloride confirmed[32] structure (2) and established the relative configuration at each of the asymmetric centres. The results are expressed by the formula, which also represents the absolute configuration.[33] Tetracycline is comparably defined[33] by formula (1) because the only molecular change involved in preparing the compound from aureomycin is replacement of chlorine by hydrogen. Similarly, the results for aureomycin apply[33] to 7-bromotetracycline.

In the case of terramycin, the gross structure was again confirmed by *X*-ray studies but these did not define the relative stereochemistry at C(5). The formulation given, (3), in which the hydroxyl group at C(5) is *trans* to that at C(6), is supported by n.m.r. evidence[34,35] and by a re-examination of the early *X*-ray

[23] M. W. Miller and F. A. Hochstein, *J. Org. Chem.*, 1962, **27**, 2525.
[24] F. A. Hochstein, M. Schach von Wittenau, F. W. Tanner, jun., and K. Murai, *J. Amer. Chem. Soc.*, 1960, **82**, 5934.
[25] B.P. 863, 419; U.S.P. 3,226,435.
[26] J. R. D. McCormick, P. A. Miller, J. A. Growich, N. O. Sjolander, and A. P. Doerschuk, *J. Amer. Chem. Soc.*, 1958, **80**, 5572.
[27] M. Schach von Wittenau, F. A. Hochstein, and C. R. Stephens, *J. Org. Chem.*, 1963, **28**, 2454.
[28] A. I. Scott and C. T. Bedford, *J. Amer. Chem. Soc.*, 1962, **84**, 2271.
[29] F. A. Hochstein, C. R. Stephens, L. H. Conover, P. P. Regna, R. Pasternack, P. N. Gordon, F. J. Pilgrim, K. J. Brunings, and R. B. Woodward, *J. Amer. Chem. Soc.*, 1953, **75**, 5455.
[30] C. R. Stephens, L. H. Conover, R. Pasternack, F. A. Hochstein, W. T. Moreland, P. P. Regna, F. J. Pilgrim, K. J. Brunings, and R. B. Woodward, *J. Amer. Chem. Soc.*, 1954, **76**, 3568.
[31] C. W. Waller, B. L. Hutchings, R. W. Broschard, A. A. Goldman, W. J. Stein, C. F. Wolf, and J. H. Williams, *J. Amer. Chem. Soc.*, 1952, **74**, 4981.
[32] J. Donohue, J. D. Dunitz, K. N. Trueblood, and M. S. Webster, *J. Amer. Chem. Soc.*, 1963, **85**, 851.
[33] V. N. Dobrynin, A. I. Gurevitch, M. G. Karapetyan, M. N. Kolosov, and M. M. Shemyakin, *Tetrahedron Letters*, 1962, 901.
[34] M. Schach von Wittenau, R. K. Blackwood, L. H. Conover, R. H. Glauert, and R. B. Woodward, *J. Amer. Chem. Soc.*, 1965, **87**, 134.
[35] Cf. M. Schach von Wittenau and R. K. Blackwood, *J. Org. Chem.*, 1966, **31**, 613.

data.[36] Probably all the fermentation tetracyclines have the same relative stereochemistry at asymmetric centres that are common and non-epimerizable.

The general structure, exemplified by formulae (1), (2), and (3), shows a number of features in a simplified way. Analysis[37,38] of the pK_a values of the three antibiotics indicates that the amphoteric forms are zwitterionic, essentially as shown (5), with both charged sites on ring A. Secondly, the diketo-amide



(5)

moiety is involved in unusual keto–enol tautomerism[37,39] and it is difficult to define its status. The dimensions[32,36] of ring A (see the Figure) in the hydrochlorides of aureomycin and terramycin indicate that the double bond is delocalised and suggest that the acidic hydrogen atom is attached to the oxygen atom of the amide group, at least in the crystal. Other evidence for extensive electron de-

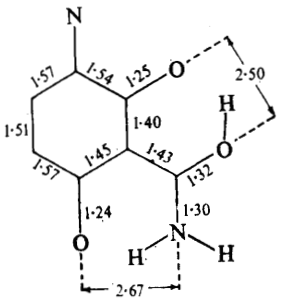


Figure *Bond distances* (Å) *in crystalline aureomycin hydrochloride*

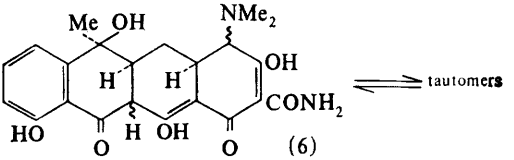
localisation is provided by the high acidity of the enolic hydrogen atom, and work with model compounds[39,40] has illustrated further the strong hydrogen bonding and highly enolic character of the system.

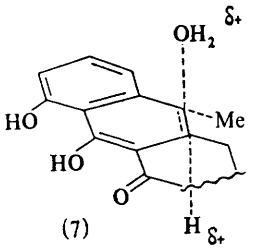
The β-diketone at C(11)—C(12) would be expected to be more stable in an enolised form[41,42] and *X*-ray measurements[32,36] show a localised double bond at C(11*a*)—C(12). The importance of this structure in solution, with respect to the alternative [double bond at C(11)—C(11*a*)] has not been reported.

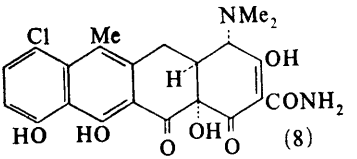
[36] H. Cid-Dresdner, *Z. Krist.*, 1965, **121**, 170.
[37] N. E. Rigler, S. P. Bag, D. E. Leyden, J. L. Sudmeier, and C. N. Reilly, *Analyt. Chem.*, 1965, **37**, 872.
[38] C. R. Stephens, K. Murai, K. J. Brunings, and R. B. Woodward, *J. Amer. Chem. Soc.*, 1956, **78**, 4155.
[39] G. O. Dudek and G. P. Volpp, *J. Org. Chem.*, 1965, **30**, 50.
[40] H. Muxfeldt, G. Grethe, and W. Rogalski, *J. Org. Chem.*, 1966, **31**, 2429.
[41] *Cf.* M. Gorodetsky, Z. Luz, and Y. Mazur, *J. Amer. Chem. Soc.*, 1967, **89**, 1183.
[42] *Cf.* H. Stetter and U. Milbers, *Chem. Ber.*, 1958, **91**, 977.

3 Reactions

The C(6) hydroxyl group of the fermentation tetracyclines together with other details of the oxygenation pattern render these materials sensitive to acids and bases [*cf.* the 6-deoxytetracyclines (p. 455)]. Consequently, reaction conditions that may be used during synthesis are limited, and the restrictions will become increasingly severe as the natural product is approached. However, the synthetic problem is simplified a little by the following observations (which are developed later). The hydroxyl group at the 12*a*-position can be replaced by a hydrogen atom and it is possible to re-hydroxylate the site in the correct stereochemical sense. It is also known that the asymmetric centre at C(4) is readily epimerised. [Tetracyclines with the unnatural configuration at C(4) have negligible bio-activity.[43]] The change is reversible, both epimers can be isolated, and no other modification takes place. In principle, therefore, synthesis of tetracycline could involve the preparation[44] of compounds of type (6).







A. Reactions in Acidic Media.—(*Cf.* the 6-deoxytetracyclines, p. 455.) The hydroxyl group at C(6) in the natural tetracyclines is secondary or tertiary. The position is benzylic and the group bears a *trans* relation to the adjacent hydrogen atom. Moreover, there is available[45] a transition state in which ring c assumes aromatic character [see (7)]. These circumstances account for the acid lability of the hydroxyl group and make dehydration to anhydro-tetracyclines [*e.g.*, anhydroaureomycin (8)] an easy process. Warm mineral acids, in aqueous or

[43] J. R. D. McCormick, S. M. Fox, L. L. Smith, B. A. Bitler, J. Reichenthal, V. E. Origoni, W. H. Muller, R. Winterbottom, and A. P. Doerschuk, *J. Amer. Chem. Soc.*, 1957, **79**, 2849.
[44] *Cf.* R. B. Woodward, *Pure Appl. Chem.*, 1963, **6**, 561.
[45] R. K. Blackwood, J. J. Beereboom, H. H. Rennhard, M. Schach von Wittenau, and C. R. Stephens, *J. Amer. Chem. Soc.*, 1963, **85**, 3943.

anhydrous solution, are usually employed for preparation of anhydro-tetracyclines,[16,17,23,31,46–48] but strong organic acids are also suitable.[46,49] The reaction

Table 1

Type of tetracycline	*Conditions*	*Temp.*	*Half-life**	*Ref.*
Tetracycline	0·2N-H_2SO_4	100°	<2 min.	*a*
7-Chloro	0·2N-H_2SO_4	100	8·2 min.	*a*
7-Bromo	0·2N-H_2SO_4	100	18·8 min.	*a*
Tetracycline	1·0N-H_2SO_4	100	⩽1 min.	*b, c*
5*a*-Epi	1·0N-H_2SO_4	100	67 min.	*c*
6-Demethyl	1·0N-H_2SO_4	100	24·8 min.	*b*
6-Demethyl	3·0N-HCl	100	1·4 min.	*d*
7-Chloro	1·0N-H_2SO_4	100	2·1 min.	*b*
7-Chloro-6-demethyl	1·0N-H_2SO_4	100	445 min.	*b*
4-Epi	1·0N-H_2SO_4	100	0·9 min.	*b*
6-Demethyl-4-epi	1·0N-H_2SO_4	100	25·8 min.	*b*
7-Chloro-4-epi	1·0N-H_2SO_4	100	5·8 min.	*b*
7-Chloro-6-demethyl-4-epi	1·0N-H_2SO_4	100	322 min.	*b*
5-Oxy	1·0N-H_2SO_4	100	4·5 min.	*b*
4-Epi-5-oxy	1·0N-H_2SO_4	100	2·6 min.	*b*
Tetracycline	1·0N-H_2SO_4	24	15·5 hr.	*e*
4-Epi	1·0N-H_2SO_4	24	24 hr.	*e*
7-Chloro	1·0N-H_2SO_4	50	7·3 hr.	*e*
7-Chloro-4-epi	1·0N-H_2SO_4	50	12·8 hr.	*e*
5-Oxy†	1·0N-H_2SO_4	50	6·3 hr.	*e*
4-Epi-5-oxy†	1·0N-H_2SO_4	50	6·0 hr.	*e*
7-Chloro	**	R	14 day	*f*
5-Oxy	Buffer/pH 1	37	114 hr.	*g*
5-Oxy	Buffer/pH 2·5	37	134 hr.	*g*
5-Oxy	Buffer/pH 4·6	37	45 hr.	*g*
5-Oxy	Buffer/pH 5·5	37	45 hr.	*g*
5-Oxy	Buffer/pH 7·0	37	26 hr.	*g*

* The results are approximate; they were obtained by measuring changes in bio-activity. Aqueous solutions were used throughout and products were not isolated.
† The anhydro-derivatives are degraded even under these conditions.
** Solution of hydrochloride (pH 2·5—2·8); R = room temperature.
[a] Ref. 19; [b] Ref. 21; [c] Ref. 26; [d] J. R. D. McCormick, E. R. Jensen, P. A. Miller, and A. P. Doerschuk, *J. Amer. Chem. Soc.*, 1960, **82**, 3381; [e] Ref. 43; [f] Ref. 1a, p. 782; [g] P. P. Regna and A. I. Solomons, *Ann. New York Acad. Sci.*, 1950, **53**, 229.

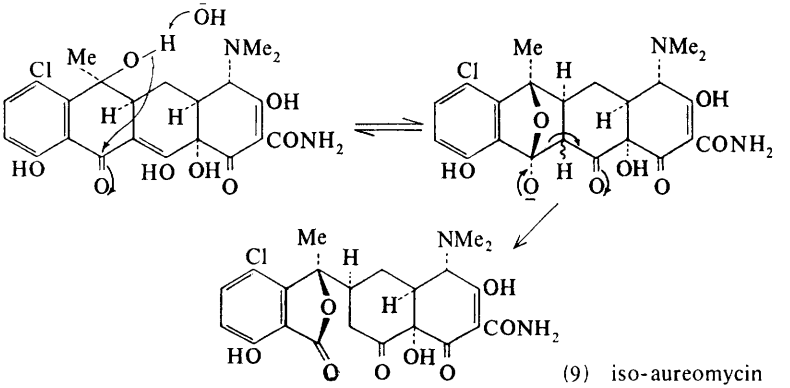
[46] R. K. Blackwood and C. R. Stephens, *Canad. J. Chem.*, 1965, **43**, 1382.
[47] J. J. Hlavka and H. M. Krazinski, *J. Org. Chem.*, 1963, **28**, 1422, footnote 4*a*.
[48] A. Green, R. G. Wilkinson, and J. H. Boothe, *J. Amer. Chem. Soc.*, 1960, **82**, 3946.
[49] B.P. 785,047.

is successful with a wide variety of tetracyclines, including those modified in ring A (see p. 449) but is sometimes complicated[50] by epimerisation at C(4). Tetracycline itself is stable for long periods in 0·03N-hydrochloric acid at room temperature, but degradation occurs if the pH is lower.[43] Further data are collected in Table 1.

The comparative stability of 6-demethyltetracycline and of the partially synthetic compound, 5*a*-epitetracycline, is not unexpected, for in one case the hydroxyl group is secondary and in the other it is *cis* to the adjacent hydrogen atom. What is noticeable, however, is the influence of a halogen at C(7). The effect is probably a steric one[32] and in the case of the 6-demethyltetracyclines it is very pronounced. The entries show that 7-chloro-6-demethyltetracycline (which is a fermentation product) can withstand acid conditions of comparative severity, and the same may be true of immediate precursors in synthesis. This exception apart, only mild conditions can be tolerated for any length of time. Table 1 also shows that the orientation of the dimethylamino-group does have some effect, but the published results are inconsistent. Those for the lower temperature range are likely to be the more reliable.

B. Reactions in Basic Media.—(*Cf.* the 6-deoxytetracyclines, p. 455.) The tetracyclines are also very sensitive to bases and data are summarised in Table 2. Again, a number of trends are apparent.

The absence of a methyl group from C(6) greatly improves base-stability and the presence of a halogen at C(7) also has a strong effect. Tetracycline is more base-resistant than aureomycin; but, in the 6-demethyl series, the chlorinated compound is the more stable. The results also suggest that it might be preferable to work with certain compounds in their 4-epi-modification and to epimerise them at a later stage.



(9) iso-aureomycin

Scheme 1

The initial products of base degradation are the isotetracyclines [*e.g.*, iso-aureomycin (9)] and they are formed as shown in Scheme 1 (ionisation of acidic groups not shown in the Scheme. An alternative process takes place in triethyl-

[50] U.S.P. 2,990,426.

Table 2

Type of tetracycline	*Conditions*	*Temp.**	*Half-life†*	*Ref.*
Tetracycline	Buffer/pH 10	22°	>600 min.	*a*
7-Chloro	Buffer/pH 10	22	18·6 min.	*a*
7-Bromo	Buffer/pH 10	22	10·9 min.	*a*
Tetracycline	0·1N-NaOH	100	6·8 min.	*b*
5-Oxy	0·1N-NaOH	100	2·2 min.	*b. c*
6-Demethyl	0·1N-NaOH	100	32 min.	*b. c*
7-Chloro	0·1N-NaOH	100	<0·3 min.	*b*
7-Chloro-6-demethyl	0·1N-NaOH	100	40 min.	*b*
Tetracycline	0·1N-NaOH	60	101 min.	*d*
4-Epi	0·1N-NaOH	60	225 min.	*d*
7-Chloro	Buffer/pH 8·8	29	53 min.	*d*
7-Chloro-4-epi	Buffer/pH 8·8	29	154 min.	*d*
5-Oxy	0·1N-NaOH	23	10·9 hr.	*d*
4-Epi-5-oxy	0·1N-NaOH	23	21·8 hr.	*d*
Tetracycline	0·1N-NaOH	25	30—34 hr.	*e*
Tetracycline	5% $NaHCO_3$	R	24—30 hr.	*f*
7-Chloro	5% $NaHCO_3$	R	1— 3 hr.	*f*
Tetracycline	Buffer/pH 8·85	R	*ca.* 12 hr.	*g*
7-Chloro	pH 8·5	R	4 hr.	*h*
5-Oxy	Buffer/pH 7	37	26 hr.	*i*
5-Oxy	Buffer/pH 8·5	37	33 hr.	*i*
5-Oxy	Buffer/pH 10	37	14 hr.	*i*

* R = Room temperature.
† The results are approximate; they were obtained by measuring changes in bio-activity. Aqueous solutions were used throughout and products were not isolated.
[a] Ref. 19; [b] Ref. 21; [c] Table 1, ref. *d*; [d] Ref. 43; [e] U.S.P. 3,122,578; [f] Ref. 30; [g] Ref. 18; [h] Ref. 1a, p. 782; [i] Table 1, ref. *g*.

amine[51] under reflux). In the case of terramycin the hydroxyl group on ring B permits further degradation, and isoterramycin has not been reported.

C. Formation of Metal Complexes.—It might be expected that degradation by alkali would be slower if the β-dicarbonyl system at C(11)—C(12) were stabilised by chelation. The tetracyclines, indeed, form complexes with many metal cations[52] and the complexes do show enhanced alkaline stability. However, the site of complex-formation remains to be settled. Model compounds representing

[51] J. S. P. Schwarz and H. E. Applegate, *J. Org. Chem.*, 1967, **32**, 1241.
[52] L. Z. Benet and J. E. Goyan, *J. Pharm. Sci.*, 1965, **54**, 983, and refs. therein.

ring A or the BCD-system are themselves chelating agents,[53,54] but these two sections of the tetracycline structure are not completely insulated by the tetrahedral 12*a*-carbon atom for there is a large degree of interaction owing to extensive intramolecular hydrogen bonding.[37] Early work drew attention to the effect of metal ions on the ultraviolet spectrum, specifically the band at *ca.* 370 mμ, of terramycin with the corresponding effect of the ions on model compounds, and led to the suggestion that the C(11)—C(12) system is the major site of complex-formation.[54]

Spectroscopic measurements on solid complexes[55] indicate that the molecules co-ordinate through oxygen, and there is some additional evidence from solution studies that the dimethylamino-group is not involved.[56]

Another approach,[57] requiring potentiometric titration of tetracyclines in the presence and absence of metal cations, has also been published; unfortunately, the results are difficult to interpret. Recent work has emphasised the fact that the macroscopic pK_a's observed for tetracyclines, *e.g.*, the values for tetracycline hydrochloride in aqueous solution are[38] *ca.* 3·3; 7·7; 9·7, each represents ionisation at more than one position on the molecule.[37,58,59] Probably, more than one site is co-ordinated both in the solid and solution phases (the nature of tetracycline complexes in solution depends on the pH[60]), and the nature of the cation itself could be a deciding structural factor. The matter is important because it is linked to the mode of action of the antibiotics; however, what is to be noted here is the established fact that complex-formation suppresses the reactions that normally take place in basic media.

A number of high-molecular-weight complexes have been prepared[61,62] of general formula (tetracycline group antibiotic) (aluminium)$_a$(calcium)$_b$(gluconic acid)$_c$ (no comment on the ionic status of the components is implied by this formulation) where *a*, *b*, and *c*, the molar ratios of the respective constituents, can vary over a wide range. Greatly enhanced alkaline stability occurs when *a* and *b* have certain values (Table 3). The molar ratio of gluconic acid appears to play an important part only in solubilising the derivative.

Of greater interest is the use[63] of concentrated ammonia solution (28% w/w) in the presence of magnesium chloride to hydrolyse the diester (10; R = Ac) to the mono-ester (10; R = H). In the absence of the metal cation degradation is 'significant' after about 1 hr., and the procedure described actually calls for a

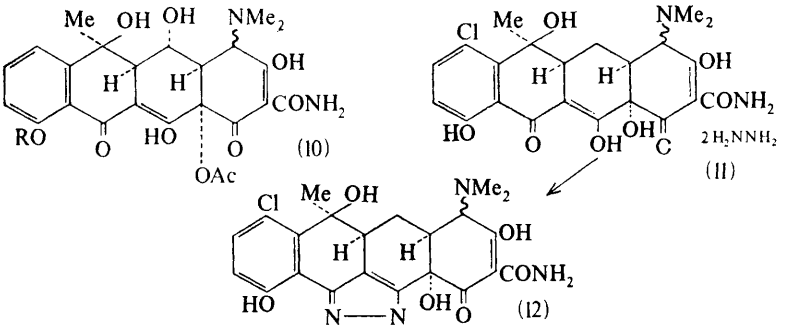
[53] J. L. Colaizzi, A. M. Knevel, and A. N. Martin, *J. Pharm. Sci.*, 1965, **54**, 1425.
[54] L. H. Conover, in 'Symposium on Antibiotics and Mould Metabolites', *Chem. Soc. Special Publ.*, No. 5, 1956, p. 48.
[55] W. A. Baker, jun., and P. M. Brown, *J. Amer. Chem. Soc.*, 1966, **88**, 1314.
[56] F. Z. Benet and J. E. Goyan, *J. Pharm. Sci.*, 1966, **55**, 1184.
[57] J. T. Doluisio and A. N. Martin, *J. Medicin. Chem.*, 1963, **6**, 16.
[58] L. J. Leeson, J. E. Krueger, and A. Nash, *Tetrahedron Letters*, 1963, 1155.
[59] K. K. Kalnin'sh and B. G. Belen'kii, *Proc. Acad. Sci.* (*U.S.S.R.*), 1964, **157**, 721.
[60] A. Albert, *Nature*, 1953, **172**, 201.
[61] E. G. Remmers, G. M. Sieger, A. P. Doerschuk, L. Ritter, and J. F. Weidenheimer, *J. Pharm. Sci.*, 1962, **51**, 86.
[62] *Cf.* E. G. Remmers, W. C. Barringer, G. M. Sieger, and A. P. Doerschuk, *J. Pharm. Sci.*, 1964, **53**, 1453.
[63] U.S.P. 3,047,617.

Table 3

Compound	*Conditions*	*Temp.*	*Half-life*	*Ref.*
7-Chloro-6-demethylTC*	0·1N-NaOH	90—100°	0·67 hr.	*a*
7- Chloro-(6-demethylTC)(Al)(Ca)(Glu)† 1 :4 :2 :12	0·1N-NaOH	90—100	50 hr.	*a*

* 7-Chloro-6-demethyltetracycline.
† No comment on the ionic status of the components is implied by this formulation. Glu = gluconic acid. [a] Ref. 61.

reaction time of 5 min. With magnesium chloride, however, a reaction time of 5 hr. is specified.

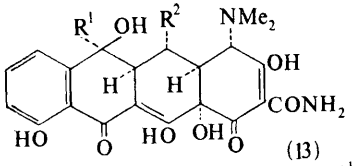


It is also relevant that tetracycline forms addition products with hydrazine[64] and can be recovered by the action of water. Aureomycin, on the other hand, cannot be regenerated from its (single) addition compound because the latter is too readily converted into a pyrazoline[(11) → (12)]. [The configuration at C(4) is probably α in compound (11). The tautomer shown (12) is likely to be more important than that given in the literature.[64]]

Although the C(6) hydroxyl group plays a central role in reactions brought about by acids and bases it also participates in a number of non-degradative transformations which temporarily modify its own activity as well as that of other positions on the tetracycline skeleton. These reactions are examined next.

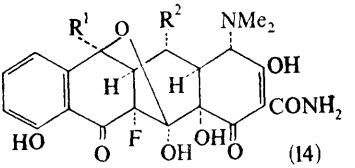
D. 6,12-Hemiketals.—When the amphoteric compounds specified by formula (13), as well as certain others of less interest, are treated with perchloryl fluoride ($FClO_3$) in the presence of a base (at least one equiv. of base per mole of substrate is required; the experiment is done with ice-bath cooling), there are obtained crystalline products for which the 11*a*-fluoro-6,12-hemiketal structures (14) have been established.[45,65,66] Formation of these derivatives is sterically

[64] U. Valcavi, G. Campanella, and N. Pacini, *Gazzetta*, 1963, **93**, 916.
[65] U.S.P. 3,109,007.
[66] U.S.P. 3,165,551.

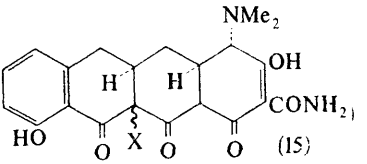


(13)

	R^1	R^2
(*a*)	Me	H
(*b*)	Me	OH
(*c*)	H	H



(14)



(15)

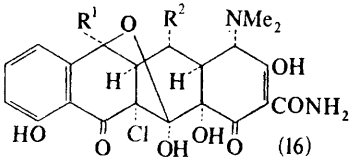
possible only if the C(5*a*) hydrogen atom and the C(6) hydroxyl group are *trans*, so that isolation of compound (14*c*) shows that the 6-demethyl-tetracyclines have the same relative stereochemistry as does the parent series.

The 11*a*-fluorine atom in 6,12-hemiketals is not especially labile, but it can be removed. In the case of (14*a*), for example, hydrogenolysis regenerates the antibiotic, though a significant amount of anhydrotetracycline is also formed. An improved yield of tetracycline (*ca.* 60% of the total product as against *ca.* 26%) is obtained by using zinc–hydrochloric acid (0·2N) for the reduction.[45,67]

When a C(6) hydroxyl group is absent (see p. 455) a hemiketal cannot form and compounds such as (15; X = F) are produced instead.[45,67] It is noteworthy that the 11*a*-fluoro-hemiketals (14) resist the usual dehydrating action of acids and can even survive treatment with boiling methanolic hydrogen chloride.[45,67] Their stability is attributed to the fluorine atom, which prevents enolisation of the C(11) carbonyl group.

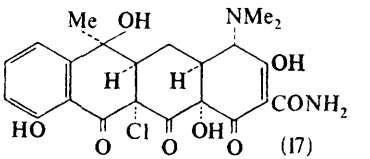
Treatment of amphoteric tetracyclines with one equivalent of *N*-chlorosuccinimide in glyme (ethylene glycol methyl ether) affords[45,65] analogous chloro-hemiketals (16) (use of two equiv. leads to 7,11*a*-dichloro-hemiketals).

Again, in the 6-deoxy-series, hemiketal formation is impossible so that the



(16)

	R^1	R^2
(*a*)	Me	H
(*b*)	Me	OH
(*c*)	H	H

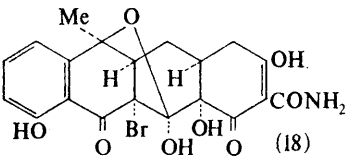


(17)

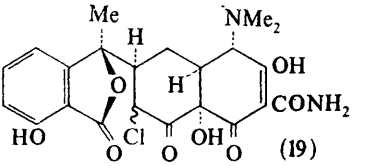
[67] H. H. Rennhard, R. K. Blackwood, and C. R. Stephens, *J. Amer. Chem. Soc.*, 1961, **83**, 2775.

product[68] is of type (15; X = Cl). The 11*a*-bromo- and 11*a*-iodo-analogues, of both series, prepared similarly, are sensitive compounds.

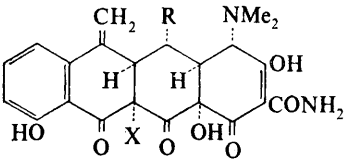
Unlike its fluoro-analogue, compound (16*a*) exists in solution partly as the C(12) ketonic form (17). [Probably the same is true of compounds (16*b*) and (16*c*), though spectroscopic evidence has not been reported.] In this tautomer, as with other compounds [*e.g.*, (15)] having a carbonyl group at C(12), the halogen atom is activated and removable by reduction under mild conditions.[45,67,68] Catalytic reduction of (16*a* ≡ 17) yields a mixture of about equal parts of tetracycline and anhydrotetracycline.[45] Use[68] of sodium dithionite might give a better result.



(18)



(19)



(20)

	R	X
(*a*)	H	Cl
(*b*)	OH	Cl
(*c*)	H	F
(*d*)	OH	F

Interestingly, the 11*a*-bromo-4-dedimethylamino-6,12-hemiketal (18) has been obtained *crystalline* not only as shown but also in its C(12) ketonic form.[45,48]

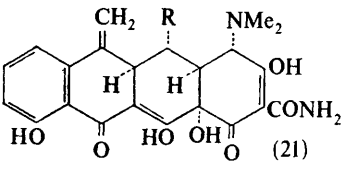
As another consequence of its tatuomeric nature, hemiketal (16*a*) does not have the acid stability of its fluoro-analogue and is converted into 11*a*-chloro-isotetracycline (19) by hot methanolic hydrogen chloride.[45]

In contrast, exocyclic dehydration takes place[45] in liquid hydrogen fluoride (hydrogen fluoride is the preferred dehydrating acid) and the reaction, which is almost instantaneous, yields the 11*a*-chloro-6-methylene derivative (20*a*). Compounds (20*b*)—(20*d*) are formed[45,66] in the same way from the corresponding 6,12-hemiketals but the rate of dehydration is lower. Consequently, it is possible to manipulate some 6,12-hemiketals in an acidic medium in order to effect substitution of ring D. In the absence of a C(6)-methyl group, 11*a*-halogeno-6,12-hemiketals are even more stable to acids so that substitution of ring D poses fewer problems. Halogenation (*N*-halogenosuccinimide–liquid hydrogen fluoride) takes place at C(7), while nitration (potassium nitrate–liquid hydrogen fluoride) occurs predominantly at C(9). The following sequence[65] is typical of those reported (no yield is given): 7-Chloro-6-demethyltetracycline was converted into 7,11*a*-dichloro-6-demethyltetracycline-6,12-hemiketal (*N*-chlorosuccinimide–glyme) and nitrated at C(9). Reduction (sodium dithionite) afforded 9-amino-7-chloro-6-demethyltetracycline.

[68] R. K. Blackwood, J. J. Beereboom, H. H. Rennhard, M. Schach von Wittenau, and C. R. Stephens, *J. Amer. Chem. Soc.*, 1961, **83**, 2773.

The 6-methylene derivatives have a much greater acid stability than the natural tetracyclines and can, therefore, undergo acid-catalysed reactions[66,69] of the type referred to for the hemiketals.

11*a*-Halogeno-6-methylene compounds are easily reduced[45] to the corresponding 6-methylene-tetracyclines [*e.g.*, (21)], zinc–mineral acid being the preferred reagent for removal of an 11*a*-fluorine atom.[65,66] The exocyclic course of the dehydration has been attributed to the presence of the 11*a*-substituent. By preventing aromatisation of ring C it witholds a powerful driving force for 5*a*,6-dehydration.[45] The acid stability of the 11*a*-halogeno-6-methylene compounds (20) is understandable on this basis, but the explanation is incomplete

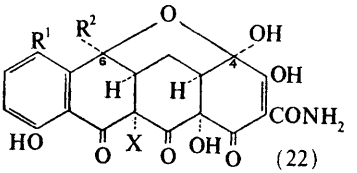


(21)

R

(*a*) H

(*b*) OH



(22)

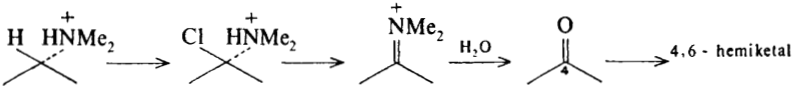
	R^1	R^2	X
(*a*)	H	Me	H
(*b*)	H	Me	Cl
(*c*)	Cl	H	H
(*d*)	Br	H	H

because the halogen-free compounds (21) also show enhanced acid-stability. They are intermediate in this respect between tetracyclines and 6-deoxy-tetracyclines (see p. 455).

E. 4,6-Hemiketals.—The hemiketals of the previous section were prepared from *amphoteric* substrates. A slight change, however, affords an entirely different series of compounds.[46,70,71] For example, treatment of an aqueous solution of tetracycline *hydrochloride* with *N*-chlorosuccinimide precipitates 4-dedimethylamino-4-oxotetracycline-4,6-hemiketal (22*a*), a substance also known as 4-hydroxy-6-methyltetracycloxide. [The presence of a little hydrochloric acid is beneficial. Other oxidising agents have been used,[71,72] but an acid must be present to bind the dimethylamine as it is released and the presence of water is essential.[73]]

Similar treatment of the 6,12-hemiketal (16*a*) in the presence of hydrochloric acid affords the new derivative (22*b*). The reaction is believed to proceed by the stages of Scheme 2, and this mechanism[46,72] is supported by the successful trapping of a 4-*NN*-dimethyliminium intermediate.[72]

[69] B.P. 995,032.
[70] R. K. Blackwood and C. R. Stephens, *J. Amer. Chem. Soc.*, 1964, **86**, 2736.
[71] U.S.P. 3,247,226.
[72] R. C. Esse, J. A. Lowery, C. R. Tamorria, and G. M. Sieger, *J. Amer. Chem. Soc.*, 1964, **86**, 3874.
[73] J. S. P. Schwarz, H. E. Applegate, J. L. Bouchard, and F. I. Weisenborn, *J. Org. Chem.* 1967, **32**, 1238.

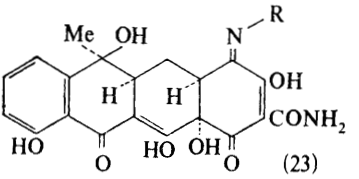


Scheme 2

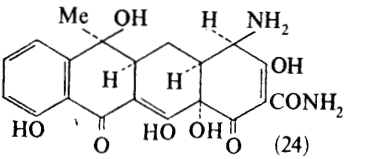
The structures (22), which were deduced from chemical experiments, demand the stereochemistry shown, and *X*-ray data[74] for the 7-halogeno-derivatives[71,72,75] confirm the assignment and indicate an unusual situation in ring A. It appears that the chloro-compound (22*c*) is enolized with a double bond at C(2)—C(3) but that the bromo-analogue (22*d*) has the double bond at C(1)—C(2).

The tetracycloxides have been examined in detail and some important transformations are established.

Hydrazine and hydroxylamine react at room temperature with tetracycloxide (22*a*) to produce the hydrazone (23*a*) and the oxime (23*b*), respectively, and both products[46] can be reduced to the 4-epi-compound (24). The same material is obtained[46] directly from tetracycloxide (22*a*) by hydrogenation in dimethylformamide containing ammonium hydroxide and magnesium chloride. In another study, compound (25) was hydrogenated in the presence of various primary amines.[72,76] A slight excess of amine provided the requisite alkaline



(23)



(24)

R

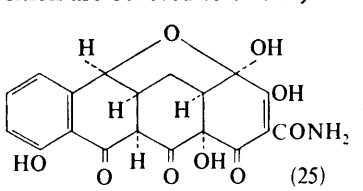
(*a*) NH_2
(*b*) OH

conditions but promoted destruction of the starting material. As a result, the products [*e.g.*, (26; R^1 = H; R^2 = Me or Et)] were obtained only in 20—40% yield. Certain of these secondary amines were alkylated reductively and then epimerised.[76] In this manner (25) was reconverted into 6-demethyltetracycline by way of intermediates (26; R^1 = H; R^2 = Me) and (26; R^1 = R^2 = Me).

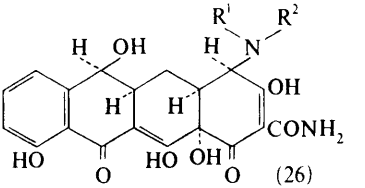
F. Photo-oxygenation.—Although the C(6)-hydroxyl group survives many reactions which involve changes at various positions of the tetracycline skeleton it might be desirable to introduce the group in the last stage of synthesis. The feasibility of such an approach was established by the discovery[28,77] that

[74] J. H. van den Hende, *J. Amer. Chem. Soc.*, 1965, **87**, 929.
[75] U.S.P. 3,159,675.
[76] R. C. Esse, J. A. Lowery, C. R. Tamorria, and G. M. Sieger, *J. Amer. Chem. Soc.*, 1964, **86**, 3875.
[77] M. Schach von Wittenau, *J. Org. Chem.*, 1964, **29**, 2746.

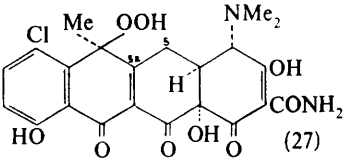
anhydroaureomycin reacts with oxygen in the presence of light to give the hydroperoxide (27). (Tautomers with the internal double bond in the 5,5*a*-position are believed to exist.[77])



(25)



(26)



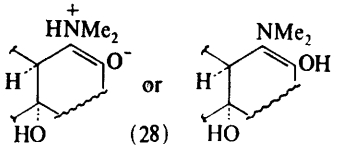
(27)

Hydrogenation cleaves the peroxide (C—O—OH → C—OH) and then effects removal of the chlorine atom and saturation of the internal double bond. The last process takes place from both faces of the molecule so that a mixture of tetracycline and its 5*a*-epimer is formed. The photoreaction is accelerated by traces of 3,4-benzopyrene and works well in the cases that have been examined except when a hydrogen atom is at C(7). In this instance, ring D is probably attacked for anhydrotetracycline gave no crystalline product, although the presence of some tetracycline was detected chromatographically.[77,78] This result is disappointing because anhydrotetracycline has been synthesised[79] so that the reaction would complete the first total synthesis of the antibiotic.

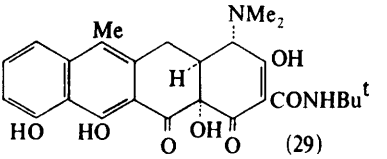
G. Modifications to Ring A.—Reversible epimerisation at C(4), mentioned earlier, has been carried out on numerous compounds.[43] The process occurs in a variety of solvent-systems, normally within the pH range 2—6, and is accelerated[43,80] by certain anions such as citrate, phosphate, or acetate. Below pH 2 and above pH 9 the rate is extremely low.[43] The technique is successful with most fermentation tetracyclines and their 6-deoxy- (see p. 455)[81] and 5*a*,6-anhydro-derivatives,[43] although tedious separation methods may be required. A more convenient procedure, reported[76,82] for a few tetracyclines, involves preparation of a metal complex *in situ* (usually with calcium ions) and adjustment of the pH to 8·5—10·0 Under these conditions 4-epi-compounds are isomerised to products of normal configuration; apparently, the reverse process does not occur to any significant extent.

[78] *Cf.* Belg. P. 631,118.
[79] A. I. Gurevitch, M. G. Karapetyan, M. N. Kolosov, V. G. Korobko, V. V. Onoprienko, S. A. Propravko, and M. M. Shemyakin, *Tetrahedron Letters*, 1967, 131.
[80] E. G. Remmers, G. M. Sieger, and A. P. Doerschuk, *J. Pharm. Sci.*, 1963, **52**, 753.
[81] Table 1, ref. *d*.
[82] U.S.P. 3,009,956.

Epimerisation involves an enolic tautomer such as (28), with a double bond at C(3)—C(4). It is understandable, because of electron delocalisation in ring A, that changes at the site of the carboxyamide group should affect the ease of the reaction. Attempts to epimerise 2-acetyl-2-decarboxyamidotetracyclines[24,83] and some tetracyclino-nitriles (formed by dehydration of the carboxyamide group; see p. 454)[43] failed so that it appears necessary for the carboxyamide group to be intact. Some modification is permissible, however, for the t-butyl derivative (29) undergoes the changes in ultraviolet absorption that characterise equilibration of the C(4) epimers of anhydrotetracycline.[84]



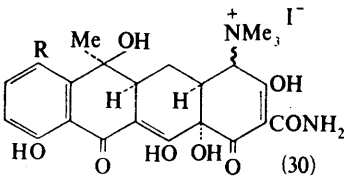
(28)



(29)

The extent of alkylation of the C(4)-nitrogen atom is also an important factor. Primary and secondary amino-groups are not epimerisable[76] (at least under normal conditions; for a possible exception see ref. 46) but tertiary amino-systems, involving a range of alkyl groups, are readily epimerised.[76]

In addition to the conformational changes, the dimethylamino-group can also be removed. One procedure, discovered with tetracycline and aureomycin, calls initially for prolonged treatment of the antibiotics with methyl iodide. Crystalline products (30*a*) and (30*b*) are obtained, though in the case of terramycin general decomposition occurs.[85] Tetracycline methiodide has the α-con-

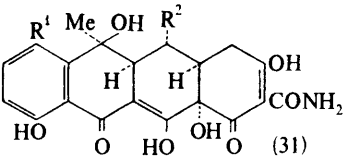


(30)

R

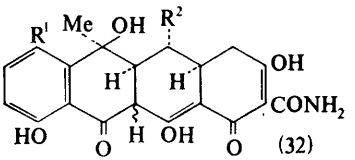
(*a*) H

(*b*) Cl



(31)

	R^1	R^2
(*a*)	H	H
(*b*)	Cl	H
(*c*)	H	OH



(32)

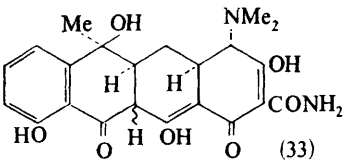
	R^1	R^2
(*a*)	Cl	H
(*b*)	H	OH
(*c*)	H	H

[83] J. Keiner, R. Hüttenrauch, and W. Poethke, *Arch. Pharm.*, 1967, **300**, 840.
[84] Ref. 43, footnote 16.
[85] J. H. Boothe, G. E. Bonvincino, C. W. Waller, J. P. Petisi, R. W. Wilkinson, and R. B. Broschard, *J. Amer. Chem. Soc.*, 1958, **80**, 1654.

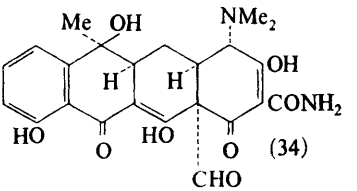
figuration at C(4) and is readily epimerised.[43] Presumably, the same is true of aureomycin methiodide. When compounds (30*a*) and (30*b*) are each treated briefly with zinc dust in aqueous acetic acid the dedimethylamino-tetracyclines (31*a*) and (31*b*) are obtained in good yield.[85] This method is also applicable to 6-deoxytetracyclines.[86]

All the dedimethylamino-compounds (31) have been produced directly from the natural products,[87] though in poor yield,[29,30,85] by prolonged action of zinc in aqueous acetic acid. In this method the by-products are[85] the dedimethylamino-12*a*-deoxy-tetracyclines (32) [other tautomers are possible; in methanolic hydrogen chloride (0·01N) it has been established[88] that C(11*a*) is tetrahedral] and by increasing the reaction time still further these are obtained as major products.[29,30,89,90]

Two methods are established for selective removal of the 12*a*-hydroxyl group. One of these requires[91,92] the use of zinc dust in dilute ammonium hydroxide and in the case of tetracycline, for example, affords the 12*a*-deoxy-compound (33) in 42% yield. This compound has a time-variable ultraviolet absorption spectrum [methanolic hydrogen chloride (0·01N)] owing to equilibration of tautomers.[93] The orientation of the dimethylamino-group has not been settled. In one case,[94] rehydroxylation at C(12*a*) gave the biologically inactive compound,



(33)



(34)

4-epitetracycline, but other experiments[95] appear to have given the active (normal) epimer. It has been observed[96] that 12*a*-deoxytetracyclines are far more resistant to epimerisation than their parent antibiotics.

The other procedure[93,96,97] involves preparation of a 12*a*-*O*-acyl derivative, usually the *O*-formate [*e.g.*, (34)]. This derivative is obtained by the action of acetic–formic anhydride[98] in pyridine [with terramycin the reaction is more

[86] C. R. Stephens, J. J. Beereboom, H. H. Rennhard, P. N. Gordon, K. Murai, R. K. Blackwood, and M. Schach von Wittenau, *J. Amer. Chem. Soc.*, 1963, **85**, 2643.
[87] U.S.P. 2,786,077.
[88] H. Muxfeldt, W. Rogalski, and K. Striegler, *Chem. Ber.*, 1962, **95**, 2581.
[89] Ref. 88, footnote 23.
[90] *Cf.* T. L. Fields, A. S. Kende, and J. H. Boothe, *J. Amer. Chem. Soc.*, 1960, **82**, 1250.
[91] Belg. P. 572,382.
[92] A. Green and J. H. Boothe, *J. Amer. Chem. Soc.*, 1960, **82**, 3950.
[93] R. K. Blackwood, H. H. Rennhard, and C. R. Stephens, *J. Amer. Chem. Soc.*, 1960, **82**, 5194.
[94] H. Muxfeldt, G. Buhr, and R. Bangert, *Angew. Chem. Internat. Edn.*, 1962, **1**, 157.
[95] U.S.P. 3,043, 877.
[96] U.S.P. 3,043,876.
[97] U.S.P. 3,002,021.
[98] L. F. Fieser and M. Fieser, 'Reagents for Organic Synthesis', J. Wiley and Sons, New York, 1967, p. 4.

complicated[93] but other 12*a*-*O*-acyl derivatives can be used;[97,99] during some acylations the C(4) position is epimerised] and is then hydrogenolysed [a C(7)-halogen atom is lost in the process[96]] under mild conditions [*e.g.*, (34) → (33)]. Again, the stereochemistry at C(4) has not been established.

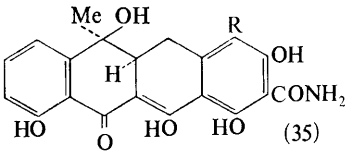
The 12*a*-position in the deoxy-compounds is an activated site. Treatment of dedimethylamino-12*a*-deoxytetracycline with one equivalent of *N*-bromosuccinimide affords the 12*a*-ξ-bromo-derivative.[48] More significantly, a hydroxyl group can be re-introduced at C(12*a*) in the correct stereochemical sense. [Strong evidence for the desired stereochemical result at C(12*a*) has not always been published; the C(4) position can be epimerised if necessary.] Microbiological hydroxylation has been reported[100] for 12*a*-deoxytetracycline, but this compound as well as many of its derivatives, including 5*a*,6-anhydro-tetracyclines, can be hydroxylated by chemical oxidants.[95,101,102] Use of metals, in the elemental form or as salts, together with gaseous oxygen appears to be a very convenient procedure[94,103,104] and in one case,[105] which was examined with special care, none of the unnatural 12*a*-epimer could be detected. The molecule in question was judged, therefore, to have a β-oriented dimethylamino-group at C(4) [whose bulk shields the face of C(12*a*)]. Peracids have been used for 12*a*-epi-hydroxylation of certain compounds.[34,54,106]

H. Aromatisation.—Besides 5*a*,6-dehydration, which usually leads to naphthacenic products, other types of aromatic compound have been made from tetracyclines.

Heating 12*a*-*O*-acyl derivatives causes *cis*-elimination of a carboxylic acid and yields[93,107] products such as (35*a*) (this compound has a time-variable ultraviolet absorption spectrum[93]). Analogous compounds, lacking the dimethylamino-group, are also obtainable.[96] Treatment of 12*a*-deoxytetracycline with methyl iodide in tetrahydrofuran containing an acid scavenger, gives[93,96] the derivative (35*b*), which also has a time-variable ultraviolet spectrum. This compound is available from the same starting material by the action[92] of perbenzoic acid or, in higher yield, by treating 12*a*ξ-bromodedimethylamino-12*a*-deoxytetracycline with pyridine.[48,108] The first of these three methods involves quaternisation (and epimerisation) of the C(4) substituent, while the second proceeds by Cope rearrangement of an *N*-oxide.

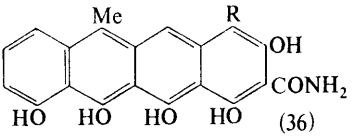
Both compounds (35*a*) and (35*b*) are fully aromatised by the action of acids

[99] U.S.P. 2,976,318.
[100] C. E. Holmlund, W. W. Andress, and A. J. Shay, *J. Amer. Chem. Soc.*, 1959, **81**, 4750.
[101] C. E. Holmlund, W. W. Andress, and A. J. Shay, *J. Amer. Chem. Soc.*, 1959, **81**, 4748.
[102] *Cf.* P. Shu, *J. Amer. Chem. Soc.*, 1966, **88**, 4529.
[103] B.P. 947,601.
[104] U.S.P. 3,188,348.
[105] R. B. Woodward, *Pure Appl. Chem.*, 1963, **6**, 561.
[106] H. Muxfeldt and A. Kreutzer, *Chem. Ber.*, 1961, **94**, 881.
[107] J. R. D. McCormick, S. Johnson, and N. O. Sjolander, *J. Amer. Chem. Soc.*, 1963, **85**, 1692, footnote 3.
[108] *Cf.* J. R. D. McCormick, J. Reichenthal, S. Johnson, and N. O. Sjolander, *J. Amer. Chem. Soc.*, 1963, **85**, 1694.



(35)

R
(*a*) NMe_2
(*b*) H

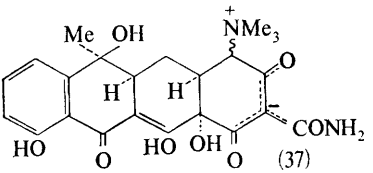


(36)

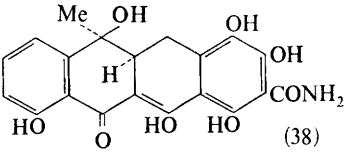
R
(*a*) NMe_2
(*b*) H
(*c*) OH

to the naphthacenes (36*a*) and (36*b*) respectively.[48,93] Compound (36*b*) is known as 6-methylpretetramid and is a biogenetic precursor[6] of tetracyclines having a methyl group at C(6).

The next stage of biogenesis is[6,9] oxidation to 4-hydroxy-6-methylpretetramid (36*c*) and this, too, can be made from tetracycline. Dissolution of tetracycline methiodide in water and adjustment of the pH to 4—5 affords [85,109] the crystalline betaine (37) and pyrolysis[110] in anhydrous acetonitrile converts this substance into the phenolic diketone (38). The latter gives 4-hydroxy-6-methylpretetramid (36*c*) on treatment with acid.[110] The thermal reaction probably

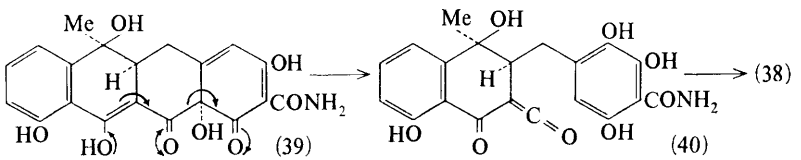


(37)

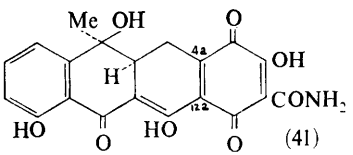


(38)

proceeds by Hofmann elimination [(37) → (39)] followed by ring cleavage [(39) → (40)]. Similar degradations have made a number of other pretetramids available.[110–112]



(39) (40) (38)



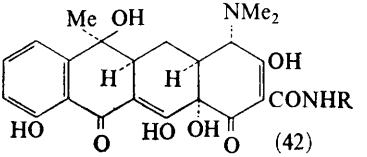
(41)

[109] *Cf.* Ref. 46 and footnote 3 therein.
[110] J. J. Hlavka, P. Bitha, and J. H. Boothe, *J. Amer. Chem. Soc.*, 1965, **87**, 1795.
[111] J. J. Hlavka, P. Bitha, and J. H. Boothe, *Tetrahedron Letters*, 1967, 1139.
[112] *Cf.* U.S.P. 3,226,305.

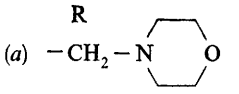
Interestingly, the A–D aromatic compound (38) has been oxidised[113] to the quinone (41). In principle, hydration of the 4*a*, 12*a*-double bond could give a tetracycloxide, bringing the sequence full circle to tetracycline.

I. Miscellaneous Reactions.—Although the carboxyamide group survives all the reactions described so far, there are of course, circumstances in which it is modified. Dehydration to a nitrile group takes place on treatment with dicyclohexylcarbodi-imide[114] or with an acid chloride in pyridine, and in the latter case esterification of the phenolic hydroxyl group at C(10) may compete with dehydration.[115]

Of pharmaceutical interest is the improved water-solubility generally conferred on a tetracycline by aminomethylation.[116–118] For example, treatment of tetracycline with morpholine and formalin yields[119] the zwitterionic and highly water-soluble compound, *N*-(morpholinomethyl)tetracycline (42*a*), apparently without epimerisation at C(4). The antibiotic can be regenerated by hydro-



(42)



genolysis over Raney nickel (43% yield) or, by treatment with aqueous sodium hydrogen sulphite (96% yield).[119] What information there is available shows aminomethyl derivatives to be subject to easy hydrolysis[120–122] to the parent antibiotic and this reaction probably accounts for their biological activity.

In an analogous type of compound, exemplified by the derivative (42*b*), the substituent on the carboxyamide nitrogen atom is probably less labile (under acidic conditions hydrolysis takes place) and has been shown to be suitable for protecting the group from dehydration.[123]

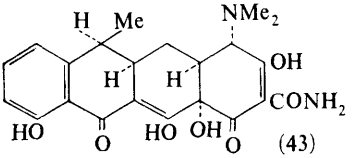
Another subject of experimental interest is the response of the tetracyclines to hydrogenation conditions. Very substantial changes can take place, but methods are available for selective reactions.

A C(7)-halogen atom (which can be a useful feature during certain stages of synthesis) can be removed cleanly by high-pressure reduction over a palladium catalyst in basic solution.[17,19,124,125]

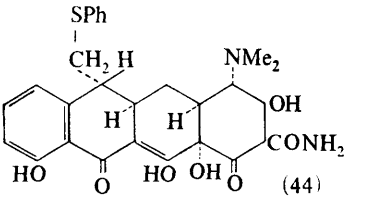
[113] J. E. Baldwin, D. H. R. Barton, L. Bould, and P. D. Magnus, *Chem. Comm.*, 1967, 319.
[114] G.P. 1,091,564.
[115] B.P. 766,512.
[116] G.P. 1,088,481.
[117] B.P. 809, 585.
[118] U.S.P. 3,104,240.
[119] W. J. Gottstein, W. F. Minor, and L. C. Cheney, *J. Amer. Chem. Soc.*, 1959, **81**, 1198.
[120] R. Hüttenrauch and J. Keiner, *Naturwiss.*, 1966, **53**, 552.
[121] A. Brunzell, *Acta Chem. Scand.*, 1962, **16**, 245.
[122] See M. J. Martell, jun., A. S. Ross, and J. H. Boothe, *J. Medicin. Chem.*, 1967, **10**, 485.
[123] C. R. Tamorria and R. C. Esse, *J. Medicin. Chem.*, 1965, **8**, 870.
[124] *Cf.* Ref. 16.
[125] G.P. 1,007,775.

Hydrogenolysis of the benzylic hydroxyl group at C(6) is also possible, and for this purpose the use of a noble-metal catalyst (usually palladium or platinum) under acidic conditions is required.[86,126] Further, addition of a chelating agent, such as boric acid, is recommended[81,126] in order to protect the C(11)—C(12)-dicarbonyl system from reduction. [A C(7) halogen atom is usually[127] lost except when a rhodium catalyst is employed in which case partial retention is observed.[128] With a rhodium catalyst a wider pH range is permissible (*e.g.*, as high as pH 9·5) and a chelating agent is not required.] The acidic conditions used do not, by themselves, promote 5*a*,6-dehydration, but the noble metal in the presence of hydrogen appears to have a catalytic effect on this process[81] and the anhydrotetracyclines produced initiate a series of competing side reactions; consequently, yields are very low. In the case of 6-demethyltetracyclines, which are more resistant to acid, yields of 30—40% have been obtained.[86,129]

Hydrogenolysis of the hydroxyl group at C(6) is accompanied by stereochemical inversion at that site;[86] therefore, tetracycline affords 6-deoxy-6β-tetracycline (43). 6-Deoxy-compounds of natural configuration are available



(43)



(44)

from the corresponding 6-methylenetetracyclines. The latter react with thiols in the presence of a free-radical initiator to give[45] compounds such as (44). Desulphurisation[86] does not disturb the stereochemistry at C(6). Alternatively, direct reduction of 6-methylenetetracyclines yields a mixture of the epimeric 6-deoxy-compounds.[69,86]

The 6-deoxytetracyclines undergo many of the reactions already discussed, such as formation of complexes and the various transformations characteristic of ring A, but in contrast to the natural tetracyclines, they are dramatically resistant to the action of acids and bases (see Table 4).

Table 4

Compound	*Conditions**	*Half-life(min.)*	*Ref.*
6-deoxy-6β-tetracycline	3N-HCl	1600	*a*
tetracycline	3N-HCl	$\ll 1$	*a*
6-deoxy-6β-tetracycline	0·1N-NaOH	570	*a*
tetracycline	0·1N-NaOH	6·8	*a*

[a] Table 1, ref. *d*. * All at 100°

[126] B.P. 855,170.
[127] *Cf.* B.P. 871,423.
[128] U.S.P. 3,019,260.
[129] U.S.P. 2,999,111.

Their enhanced stability has made possible substitution in the aromatic ring D, a process which usually requires the presence of a strong acid. [The strongly acid conditions used should not allow epimerisation at C(4); however, definite evidence for the stereochemistry has not often been sought.]

Nitration, which is often carried out in concentrated sulphuric acid, generally affords mixtures of 9- and 7-nitro-products (if both sites are available)[130–133] and the new compounds provide access to a wide range of derivatives. Reduction gives the corresponding amines[86,130,131] which can be acylated[130,131] or diazotised,[134,135] and in the latter case nucleophilic substitution of ring D becomes possible.[134]

C(9)-Diazonium compounds are useful as intermediates in the preparation of the corresponding halogeno- (fluoro-, chloro-, bromo-, iodo-) derivatives.[86,136] The methods are also applicable[136] in the C(7) series, but bromination and iodination at C(7) is achievable directly through the use of the appropriate *N*-halogeno-amide in concentrated sulphuric acid.[130,134] [Bromination studies revealed[134] that, in acetic acid, halogenation occurs at C(11*a*).] Chlorination at C(7) (*N*-chlorosuccinimide–trifluoroacetic acid) is more complicated[86] for the product contains an 11*a*-chlorine atom which must be removed, and some substitution occurs at C(9).

Besides making possible this partial synthetic work the convenient stability of 6-deoxytetracyclines has focused attention[5] on them as objectives for total synthesis and in this particular area the problems have been solved. The prototype of the series, 6-demethyl-6-deoxytetracycline has been obtained in two laboratories (as its racemate) by total synthesis[105,137] and the achievement marks a highlight in the synthetic field, for the compound, though not occurring naturally, possesses the full biological activity characteristic of the tetracycline antibiotics.

[130] J. L. Spencer, J. J. Hlavka, J. Petisi, H. M. Krazinski, and J. H. Boothe, *J. Medicin. Chem.*, 1963, **6**, 405.
[131] J. Petisi, J. L. Spencer, J. J. Hlavka, and J. H. Boothe, *J. Medicin. Pharmaceut. Chem.*, 1962, **5**, 538.
[132] J. H. Boothe, J. J. Hlavka, J. P. Petisi, and J. L. Spencer, *J. Amer. Chem. Soc.*, 1960, **82**, 1253.
[133] J. J. Beereboom, J. J. Ursprung, H. H. Rennhard, and C. R. Stephens, *J. Amer. Chem. Soc.*, 1960, **82**, 1003.
[134] J. J. Hlavka, A. Schneller, H. Krazinski, and J. H. Boothe, *J. Amer. Chem. Soc.*, 1962, **84**, 1426.
[135] J. J. Hlavka, H. Krazinski, and J. H. Boothe, *J. Org. Chem.*, 1962, **27**, 3674.
[136] B.P. 935,384.
[137] H. Muxfeldt and W. Rogalski, *J. Amer. Chem. Soc.*, 1965, **87**, 933.