Tetracycline Studies. Part 6.[1] 6- and 12a-Hydroxylation of 6-Methylpretetramid

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Oxygenation of 6-methylpretetramid (1) in dimethylformamide–aqueous alkali resulted in specific hydroxylation at the 6- and 12a-positions. The molecular structure of the product (29) was established by degradation studies and by means of spectroscopic evidence.

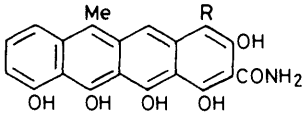
It has been established [2] that 6-methylpretetramid (1) is a biosynthetic precursor of the tetracyclines (3)—(5); enzyme-catalysed hydroxylation processes are involved in this transformation.[3] It is of interest, therefore, to study reactions that could result in the 6- and 12a-hydroxylation of 6-methylpretetramid, the synthesis of which has been achieved recently.[4]

It has been shown in studies of biosynthesis using 5a,6-anhydrotetracycline (10) that 6-hydroxylation occurs at a late stage in the formation of tetracyclines.[5] This step has been simulated chemically by oxygenation of 5a,6-anhydro-7-chlorotetracycline (11) in the presence of u.v. light and a photosensitizer.[6,7] The resulting hydroperoxide (15) was reduced in two stages to give 7-chlorotetracycline (5). The conversion of 6-methylpretetramid (1) into the quinone (16) [8,9] also involves 6-hydroxylation. Since 5a,6-anhydro-12a-deoxytetracycline (12) is transformed into 12a-deoxytetracyclines, alone, by mutant strains of *Streptomyces aureofaciens*, it is likely that 12a-hydroxylation occurs at an early stage in the transformation of 6-methylpretetramid into tetracyclines.[5]

Various reagents have been employed for the oxidation of simple alkylphenols to 4-hydroperoxy- and 4-hydroxycyclohexa-2,5-dienones or to the corresponding 6-substituted derivatives of cyclohexa-2,4-dienones, processes which may be regarded as analogous to 6- and 12a-hydroxylation of 6-methylpretetramid. Lead tetraacetate has been employed in a number of cases,[10–12] including, recently, the conversion of the ring A model (17) into the acetate (19).[13] Other reagents include thallium triacetate,[14] t-butyl peroxide,[15] benzoyl peroxide,[16] trifluoroperacetic acid,[17] and peroxosulphuric acid.[18] Transition metal ion–hydrogen peroxide systems have been employed for the oxidation of phenols. A number of trialkylphenols have been converted into the corresponding hydroperoxycyclohexadienones by titanium(IV) or molybdenum(IV) and hydrogen peroxide.[19] In an approach to the synthesis of tetracyclines which involves 12a-hydroxylation of a 4a,12a-anhydrotetracycline derivative, Barton *et al.* have employed cerium(IV) oxide–hydrogen peroxide for conversion of the model amide (18) into the hydroperoxycyclohexadienone (20), which may be reduced to the required hydroxycyclohexadienone (21).[20] The same product (21) results from treatment of (18) with diphenylseleninic anhydride.[21] There are examples of

[1] Part 5, M. J. Broadhurst, C. H. Hassall, and G. J. Thomas, *J.C.S. Perkin I*, 1977, 2502.
[2] J. R. D. McCormick, S. Johnson, and N. O. Sjolander, *J. Amer. Chem. Soc.*, 1963, **85**, 1692.
[3] J. R. D. McCormick, 'Biogenesis of Antibiotic Substances,' ed. Z. Vanek and Z Hostalek, Academic Press, New York, 1965.
[4] D. H. R. Barton, P. D. Magnus, and T. Hase, *J. Chem. Soc.* (*C*), 1971, 2215.
[5] J. R. D. McCormick, P. A. Miller, S. Johnson, N. Arnold, and N. O. Sjolander, *J. Amer. Chem. Soc.*, 1962, **84**. 3023.
[6] A. I. Scott and C. T. Bedford, *J. Amer. Chem. Soc.*, 1962, **84**, 2271.
[7] M. S. von Wittenau, *J. Org. Chem.*, 1964, **29**, 2746.
[8] C. H. Hassall and T. E. Winters, *Chem. Comm.*, 1967, 77.
[9] C. H. Hassall and T. E. Winters, *J. Chem. Soc.* (*C*), 1968, 1558.
[10] F. Wessely and F. Sinwel, *Montash.*, 1950, **81**, 1055.
[11] F. Wessely, J. Swoboda, and V. Guth, *Montash.*, 1964 **35**, 649.
[12] A. M. Gold and E. Schwenk, *J. Amer. Chem. Soc.*, 1958, **80**, 5683.
[13] D. H. R. Barton, L. Bould, D. L. J. Clive, P. D. Magnus, and T. Hase, *J. Chem. Soc.* (*C*), 1971, 2204.
[14] E. Hecker and R. Lattrell, *Angew. Chem.*, 1962, **74**, 652.
[15] T. W. Campbell and G. M. Coppinger, *J. Amer. Chem. Soc.*, 1952, **74**, 1469.
[16] S. L. Cosgrove and W. A. Waters, *J. Chem. Soc.*, 1951, 388.
[17] R. D. Chambers, P. Goggin, and W. K. R. Musgrave, *J. Chem. Soc.*, 1959, 1804.
[18] E. Bamberger, *Chem. Ber.*, 1903, **36**, 2028.
[19] R. G. R. Bacon and L. C. Kuan, *Tetrahedron Letters*, 1971, 3397.
[20] D. H. R. Barton, P. D. Magnus, and J. C. Quinney, *J.C.S. Perkin I*, 1975, 1610.
[21] D. H. R. Barton, P. D. Magnus, and M. N. Rosenfeld, *J.C.S. Chem. Comm.*, 1975, 301.

autoxidation of phenols in alkaline solution to hydroperoxycyclohexadienones. Thus the phenol (22) gives the dienone (23),[22,23] and alkaline autoxidation of the resorcinol derivative (24) gives the hydroxycyclohexadienone (25),[24] formed by alkaline decomposition of the hydroperoxide (26).[25] Somewhat similar methods have been described for hydroxylation of 12a-deoxytetracyclines *in vitro*. They include the use of peroxy-acids,[26] oxygenation in the presence of noble metal catalysts,[27] and the action of oxygen or hydrogen peroxide in the presence of transition metal ions.[28]



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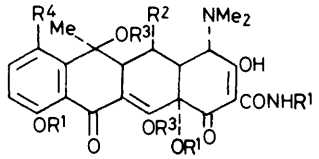
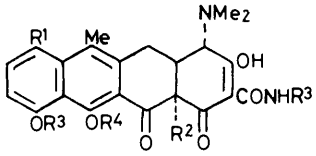
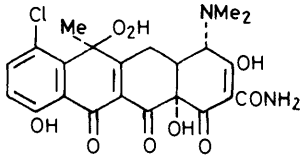
(2) R = OH



(3) $R^1 = R^2 = R^3 = R^4 = H$
(4) $R^1 = R^3 = R^4 = H$, $R^2 = OH$
(5) $R^1 = R^2 = R^3 = H$, $R^4 = Cl$
(6) $R^1 = SiMe_3$, $R^2 = R^3 = R^4 = H$
(7) $R^1 = R^3 = SiMe_3$, $R^2 = R^4 = H$
(8) $R^1 = SiMe_3$, $R^2 = OSiMe_3$, $R^3 = R^4 = H$
(9) $R^1 = R^3 = SiMe_3$, $R^2 = OSiMe_3$, $R^4 = H$



(10) $R^1 = R^3 = R^4 = H$, $R^2 = OH$
(11) $R^1 = Cl$, $R^2 = OH$, $R^3 = R^4 = H$
(12) $R^1 = R^2 = R^3 = R^4 = H$
(13) $R^1 = R^4 = H$, $R^2 = OSiMe_3$, $R^3 = SiMe_3$
(14) $R^1 = H$, $R^2 = OSiMe_3$, $R^3 = R^4 = SiMe_3$



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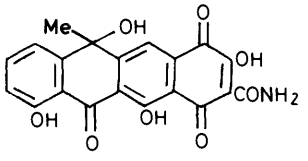

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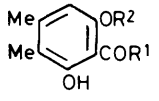
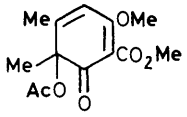


(17) $R^1 = OMe$, $R^2 = Me$
(18) $R^1 = NH_2$, $R^2 = H$



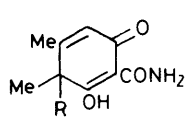
(19)

In a study using the carboxamide (27) as a model compound, Hassall and Winters showed that the quinone (28) was formed in good yield by the action of Frémy's salt in phosphate buffer.[8,29] Hydroxylation at the unsubstituted position also occurred as a result of oxygenation of the phenol (27) in alkali, but this process was complicated by side reactions involving dimerisation or elimination of the 3-acetyl group. When 6-methylpretetramid (1) was treated with Frémy's salt, oxidation occurred rapidly, leading to a complex mixture. However, oxygenation in alkali was more specific and yielded the quinone (16).[8,9] The same product was obtained through oxygenation of 4-hydroxy-6-methylpretetramid (2) in dimethyl sulphoxide–magnesium acetate solution.[30] Trial experiments using mixtures of 6-methylpretetramid (1) and the quinone (16) led to the development of a method for separation of these compounds, based on the more acidic character of the hydroxy-quinone (16).[9]

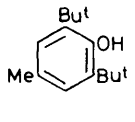
Oxygenation of 6-methylpretetramid in 0.1M-potassium hydroxide–dimethylformamide (DMF) (1 : 1) took a different course. Reaction ceased after uptake of 2 mol. equiv. of oxygen to give a good yield of a bright orange microcrystalline product to which we have assigned the structure (29). The quinone (16), the sole

[22] H. R. Gersmann and A. F. Bickel, *J. Chem. Soc.*, 1959, 2711.
[23] H. R. Gersmann and A. F. Bickel, *J. Chem. Soc.*, 1962, 2356.
[24] H. Musso, D. Maassen, and D. Bormann, *Chem. Ber.*, 1962, **95**, 2837.
[25] M. S. Kharasch and B. S. Joshi, *J. Org. Chem.*, 1957, **22**, 1439.
[26] H. Muxfeldt and A. Kreutzer, *Chem. Ber.*, 1961, **94**, 881.
[27] H. Muxfeldt, G. Buhr and R. Bangat, *Angew. Chem. Internat. Edn.*, 1962, **1**, 157.
[28] L. H. Conover, K. Butler, J. D. Johnston, J. J. Korst, and R. B. Woodward, *J. Amer. Chem. Soc.*, 1962, **82**, 3222.
[29] C. H. Hassall and T. E. Winters, *J. Chem. Soc.* (*C*), 1967, 912.
[30] J. R. D. McCormick and E. R. Jensen, *J. Amer. Chem. Soc.*, 1965, **87**, 1794.

product of oxygenation of 6-methylpretetramid in aqueous alkali alone,[8,9] was not formed in the presence of DMF. The compound (29) was also obtained through



(20) R = O_2H
(21) R = OH



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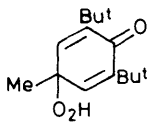

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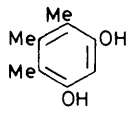

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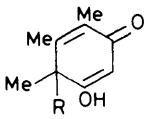
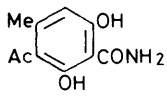


(25) R = OH
(26) R = O_2H



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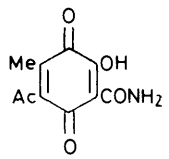

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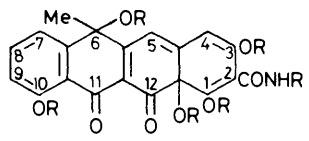
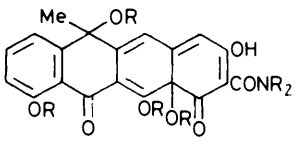
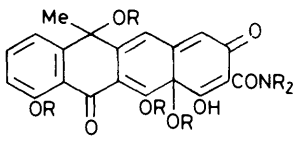


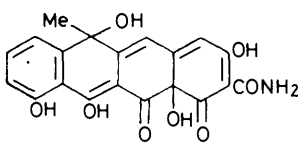
(29a) R = H
(30a) R = $SiMe_3$



(29b) R = H
(30b) R = $SiMe_3$



(29c) R = H
(30c) R = $SiMe_3$



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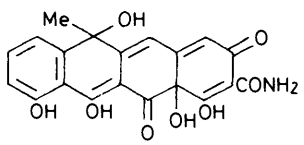


(29e)

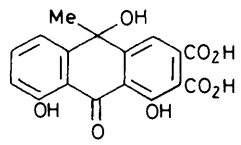
oxygenation of 6-methylpretetramid in dimethyl sulphoxide–magnesium acetate solution.[31]

Evidence for the molecular structure of the hydroxylation product (29). Combustion analysis and high resolution mass spectrometry defined the molecular formula of the major oxidation product (29) as $C_{20}H_{15}NO_8$; this was confirmed through the preparation of triacetyl and hexakistrimethylsilyl derivatives.

Treatment with hydriodic acid in phenol converted compound (29) into 6-methylpretetramid in good yield; this confirmed that the original carbon skeleton and the substituents at positions 1, 2, 3, 6, 10, 11, and 12 remained. No 4-hydroxy-6-methylpretetramid (2), the product of reduction of the quinone (16) [30] with hydriodic acid under similar conditions, was found in the reaction mixture.

When the tetracyclic compound (29) was degraded by alkaline hydrogen peroxide, the tricyclic dicarboxylic acid (31), a known product of oxidation of the quinone (16),[30] was formed; evidently compound (29) did not have oxygen substituents at positions 5, 7, 8, and 9. The 6-hydroxy-function might have been introduced into the acid (31) in the degradation process, but separate evidence precluded this. It could be shown that the compound (29) was converted into the quinone (16), which incorporated a 6-hydroxy-group, by treatment with alkali alone—conditions that could not be expected to give rise to hydroxylation at position 6. Moreover, the ^1H n.m.r. evidence indicated that there was an oxygen function at position 6. The signals for the 6-methyl group in the compound itself and in the triacetyl and hexakistrimethylsilyl derivatives were at τ 8.46 [in $(CD_3)_2SO$], 8.24 [in $(CD_3)_2SO$], and τ 8.18 and 8.25 (3 H, in CCl_4), respectively. This established that the methyl group was not attached to an aromatic ring c but at the same position as the 6-hydroxy-function, as in the anthrone (31), τ 8.46 [in $(CD_3)_2SO$].

Evidence for locating the single unaccounted for hydroxy-function at position 12a was necessarily indirect since no compounds with structures immediately related



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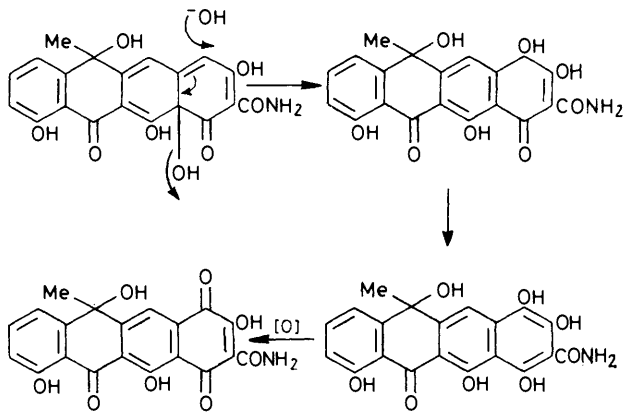

(16) (32)

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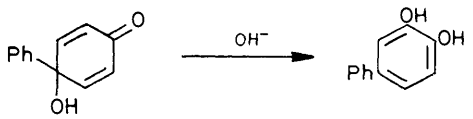
to (29) have been synthesised, no crystalline derivative suitable for *X*-ray analysis has been prepared, and no simple degradation procedure for locating such a function

[31] C. H. Hassall and G. J. Thomas, *Chem. Comm.*, 1970, 1053.

has been defined. Also, compound (29) was not sufficiently soluble in suitable solvents to allow measurement of its ^{13}C n.m.r. spectrum. However, the conversion of compound (29) into the quinone (16) by the action of alkali was informative. The reduction of (29) by hydriodic acid–phenol indicated that it had no oxygen function at position 4. The formation of the quinone (16) required, then, an explanation that took into account the location of one hydroxy-group in a structure based on the earlier evidence. We attribute this transformation to attack by hydroxide ion on the dienone (29) at position 4 to give the reduced quinone (32), which was oxidised in air to the quinone itself (Scheme). This reaction is very similar to that involved in the conversion of 4-hydroxy-4-phenylcyclohexa-2,5-dienone (33) into biphenyl-3,4-diol (34) in alkali.[32]



(33) (34)

The ^1H n.m.r. spectra of compound (29) and its derivatives provided convincing evidence of the substitution pattern of rings B, C, and D. The information relating to the 6-hydroxy-group has been described. Multiplets due to three adjacent aromatic protons were similar to those in the ring D of tetracyclines (Table 1).

TABLE 1

N.m.r. signals due to ring D protons [in $(CD_3)_2SO$]

Compound	τ (H-7)	τ (H-8)	τ (H-9)	$J_{7,8}$/ Hz	$J_{8,9}$/ Hz
Tetracycline (3)	2.95	2.53	3.16	8	8
5-Hydroxytetracycline (4)	2.86	2.46	3.11	8	8
4a,12a-Anhydrotetracycline (35)	2.74	2.37	3.17	8	8
Compound (29), major isomer	2.69	2.35	3.07	8	8
Compound (29), minor isomer	2.65	2.39	3.12	8	8

TABLE 2

Effect of *O*-trimethylsilylation on ring D proton chemical shifts

Derivative	Δν(H-7)/ Hz	Δν(H-8)/ Hz	Δν(H-9)/ Hz
Tristrimethylsilyltetracycline (6) [33]	−23	+11	−4
Pentakistrimethylsilyltetracycline (7)[33]	−17	+17	0
Tetrakistrimethylsilylterramycin (8)[33]	−17	+24	+2
Hexakistrimethylsilylterramycin (9)[33]	−24	+15	+10
Derivative (30), major isomer	−16	+15	+5
Derivative (30), minor isomer	obscured	obscured	+4

$\Delta\nu = \nu(CCl_4)$ for derivative $-\nu[(CD_3)_2SO]$ for parent.

Moreover, the effects of *O*-trimethylsilylation on the chemical shifts of these protons were very similar for the two cases (Table 2). This provided further confirmation that ring D of compound (29) and of 6-methylpretetramid had similar substituents. A detailed study of the complex multiplet in the region τ 2.2—3.5 (total 5 H) suggested that the product was a mixture of two closely related isomeric forms, in equilibrium. When the signals due to protons at positions 7, 8, and 9 had been assigned, singlets at τ 3.35 and 2.72 [in $(CD_3)_2SO$] and two other singlets of approximately half their intensity at τ 3.40 and 2.68 (total 2 H) remained unaccounted for. We have assigned these signals to protons at positions 4 and 5 in structure (29). Trimethylsilylation caused a downfield shift of the signals due to these protons; on the basis of studies with model compounds (37)—(39) and (43) (Table 3), this precluded an aromatic ring A.

TABLE 3

Effect of trimethylsilylation on chemical shifts of compound (29) and ring A models

Parent	τ[$(CD_3)_2SO$]	Derivative	τ(CCl_4)	Δν/Hz
(37)	3.46	(40)	3.63	+17
(38)	3.80	(41)	3.86	+6
(39)	3.72	(42)	3.77	+5
(43)	3.62	(44)	3.80	+18
(29), major isomer	2.72	(30), major isomer	2.63	−9
	3.35		3.21	−14
(29), minor isomer	2.68	(30), minor isomer	2.60	−8
	3.40		3.25	−15

This was confirmed by deuteriation studies involving addition of a trace of sodium deuterioxide to a solution in $CDCl_3$–$(CD_3)_2SO$ (trace). The signals at τ 3.30 and 3.34 disappeared immediately. Evidently they were due to protons in a keto–enol system. On the other hand, the singlets at τ 2.62 and 2.58 disappeared slowly (intensity 0.4 H after 16 h at 20 °C). The multiplicity of ^1H n.m.r. signals attributed to the 4- and 5-protons could arise from either tautomers or stereoisomers. The former explanation is indicated by the change in ratio of isomers on standing in $(CD_3)_2SO$ or $CDCl_3$–$(CD_3)_2SO$, in the presence of traces of sodium deuterioxide (Table 4). ^1H N.m.r. signals due to OH

TABLE 4

Ratio of isomers observed in ^1H n.m.r. spectra of (29)

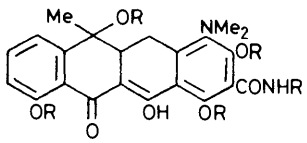
Solvent	Time (min.)	Ratio
$CDCl_3$–trace $(CD_3)_2SO$	0	2.0 : 1
$CDCl_3$–trace $(CD_3)_2SO$–trace NaOD	5	1.6 : 1
$CDCl_3$–trace $(CD_3)_2SO$–trace NaOD	90	1.4 : 1
$(CD_3)SO$	0	2.1 : 1
$(CD_3)_2SO$–trace NaOD	30	1.7 : 1
$(CD_3)_2SO$–trace NaOD	90	1.2 : 1
CCl–$(CD_3)_2SO$(4 : 1)	0	1.8 : 1

and NH provided further evidence for the tautomerism of compound (29). Signals due to these functions have been assigned in n.m.r. spectra of a number of tetracyclines [33-35] and model compounds.[36] The chemical shifts

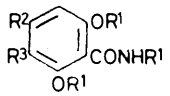
[32] Y. Abe, *Bull. Chem. Soc. Japan*, 1943, **18**, 93.
[33] C. H. Hassall and G. J. Thomas, *J. Chem. Soc.* (*C*), 1970, 636.
[34] G. L. Asleson, L. J. Stoel, E. C. Newman, and C. W. Frank, *J. Pharm. Sci.*, 1974, **63**, 1144.
[35] D. E. Williamson and G. W. Everett, jun., *J. Amer. Chem. Soc.*, 1975, **97**, 2397.
[36] G. O. Dudek and G. P. Volpp, *J. Org. Chem.*, 1965, **30**, 50.

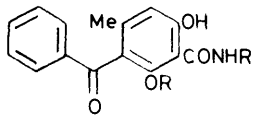
of NH and OH vary considerably with concentration, temperature, and solvent, but protons involved in intramolecular hydrogen bonding are much less susceptible to change by these factors.

The dioxo-amide system (45) of the tetracyclines gives rise to two signals in the regions τ 0.4—0.9 and 0.9—1.4 [in $(CD_3)_2SO$] (Table 5). Model studies indicate that the



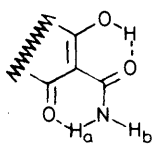
(35) R = H
(36) R = $SiMe_3$



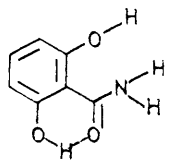


(37) $R^1 = R^2 = R^3 = H$
(38) $R^1 = R^3 = H$, $R^2 = Me$
(39) $R^1 = H$, $R^2 = Me$, $R^3 = Ac$
(40) $R^1 = SiMe_3$, $R^2 = R^3 = H$
(41) $R^1 = SiMe_3$, $R^2 = Me$, $R^3 = H$
(42) $R^1 = SiMe_3$, $R^2 = Me$, $R^3 = Ac$

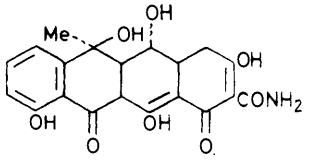
(43) R = H
(44) R = $SiMe_3$



(45)



(46)



(47)

signal at lower field is due to a proton (H_a) which is intramolecularly hydrogen bonded to the oxo-function, and whose chemical shift varies little with solvent, whereas the signal at higher field is due to a proton (H_b) which is not intramolecularly hydrogen bonded, and whose chemical shift varies widely with solvent. *N*-Trimethylsilyl derivatives of the tetracyclines show a single NH signal in the region τ 0.4—0.8 (in CCl_4).[33] In contrast, 2,6-dihydroxybenzamides (46) give rise to a single band in the region τ 1.5—2.0 (2 H) [in $(CD_3)_2SO$], and their *N*-trimethylsilyl derivatives to a band at τ 4.4—4.9 (in CCl_4) (Table 5). The amide function of tautomer (29a) is in a similar environment to structure (46), and tautomers (29b—e) resemble structure (45). The ^1H n.m.r. spectrum of compound (29) in $(CD_3)_2SO$, in which the ratio of isomers is *ca.* 2 : 1, includes signals at τ 0.4 and 0.9 (each 0.66 H). These signals are

TABLE 5

Chemical shifts of amide protons of tetracyclines and models

Parent	Amide type	$\tau(CONH_2)$ [in $(CD_3)_2SO$]	Me_3Si Derivative	$\tau(CONHSiMe_3)$ (in CCl_4)
(3)	(45)	0.88, 1.35	(6)	0.57
			(7)	0.60
(4)	(45)	0.83, 1.28	(8)	0.79
			(9)	0.78
(10)	(45)	0.80, 1.10	(13)	0.45
			(14)	0.47
(37)	(46)	1.75	(40)	4.90
(38)	(46)	2.00	(41)	4.55
(39)	(46)	1.75	(42)	4.41
(35)	(46)	1.54, 1.72	(36)	4.82

assigned to the amide function of tautomer (29b, c, d, or e) accounting for 66% of the isomeric mixture. A broad band at τ 1.4—2.2 (1.3 H) is assigned to the amide function of the minor isomer (29a) (0.66 H), together with unidentified hydroxy-groups (0.66 H). In CCl_4–$(CD_3)_2SO$ (4 : 1) a signal at τ 0.4 (0.66 H) is assigned to the intramolecularly hydrogen bonded amide proton (H_a) in tautomer (29b, c, d, or e). A signal at τ 1.8 (2 H) is assigned to the amide group of tautomer (29a) (0.66 H), the remaining amide proton (H_b) of tautomer (29b, c, d, or e) (0.66 H), and unidentified hydroxy-functions (0.66 H). The fact that the unassociated proton (H_b) has a higher chemical shift in the less polar solvent is in agreement with results obtained for model compounds.[36]

N.m.r. signals due to hydrogen-bonded hydroxy-groups could also be assigned through comparison of compound (29) with the tetracyclines. The 10-hydroxy-function of the tetracyclines is hydrogen-bonded to the 11-oxo-function and gives rise to a signal in the region τ −2 to −1.5 [in $(CD_3)_2SO$], whereas the 10-hydroxy-function of 5a,6-anhydrotetracyclines [*e.g.* (10)] is much less strongly hydrogen-bonded and resonates at τ *ca.* 0.1. The signal at τ −2.1 (1 H) [in CCl_4–$(CD_3)_2SO$] in the ^1H n.m.r. spectrum of compound (29) indicates that both tautomers present contain a 10-hydroxy-group which is hydrogen-bonded to the 11-oxo-function, precluding 11-hydroxy-forms such as (29d and e).

The n.m.r. spectrum of the hexakistrimethylsilyl derivative (30) suggested the presence of isomers related to the tautomeric forms (29a) and (29b or c), in a ratio of *ca.* 2 : 1. A signal at τ 4.76 (0.33 H) (in CCl_4) was assigned to the $CONHSiMe_3$ function of the product (30a) derived from tautomer (29a). By analogy with a number of tetracyclines,[33] in which monotrimethylsilylation of the amide group occurs, and the 3-enol function does not react, trimethylsilylation of tautomeric forms (29b) or (29c) would be expected to yield pentakistrimethylsilyl derivatives. However, integration of the

region τ 9.5—9.7 in the n.m.r. spectrum, together with mass spectral data, indicated that trimethylsilylation of compound (29) during 3 h yielded 2 isomeric hexakis-trimethylsilyl derivatives. A signal at τ −5.9 (0.66 H) could not be attributed to a $-CONHSiMe_3$ function. It was concluded that bis-*N*-trimethylsilylation had occurred to yield the derivative (30b or c), and the signal at τ −5.9 was assigned to the unchanged enolic hydroxy-group at position 3 or 1. Assignments of low-field signals in the n.m.r. spectra of compound (29) and its hexakistrimethylsilyl derivatives are summarised in Table 6.

TABLE 6

Assignments of low-field signals in ^1H n.m.r. spectra of (29) and (30)

Solvent	Isomer Ratio	τ	Int.	Assignment
$(CD_3)_2SO$	2.1 : 1	−6 to −2	2 H	1- or 3-OH in (29a) (0.33 H)
[comp. (29)]				10-OH in (29a) (0.33 H)
				10-OH in (29b) or (29c) (0.66 H)
				12-OH in (29b) or (29c) (0.66 H)
		0.4	0.66 H	$CONH_a$ in (29b) or (29c) (0.66 H)
		0.9	0.66 H	$CONH_b$ in (29b) or (29c) (0.66 H)
		1.4—2.2	1.33 H	$CONH_2$ in (29a) (0.66 H)
				Unassigned OH (0.66 H)
CCl_4–$(CD_3)_2SO$ (4 : 1)	1.8 : 1	−6.2 to −1	1 H	1 -OH or 3-OH in (29a) (0.33 H)
				12-OH in (29b) or (29c) (0.66 H)
[comp. (29)]		−2.1	1 H	10-OH in (29a) (0.33 H)
				10-OH in (29b) or (29c) (0.66 H)
		0.4	0.66 H	$CONH_a$ in (29b) or (29c) (0.66 H)
		1.8	2 H	$CONH_b$ in (29b) or (29c) (0.66 H)
				$CONH_2$ in (29a) (0.66 H)
				Unassigned OH (0.66 H)
CCl_4	1.8 : 1	−5.9	0.66 H	3-OH in (30b) or 1-OH in (30c) (0.66 H)
[comp. (30)]		4.76	0.33 H	$CONHSiMe_3$ in (30a) (0.33 H)

This indication that 6-methylpretetramid may be hydroxylated by chemical means at positions 6 and 12a encourages the prospect of devising a synthesis of tetracyclines which simulates the biosynthetic route.

EXPERIMENTAL

M.p.s were determined on a Kofler hot-stage apparatus. I.r. spectra were recorded on a Perkin-Elmer 257 spectrophotometer for KBr discs, unless otherwise stated. U.v. and visible spectra were determined with a Unicam SP 800 spectrophotometer; log ε values have an accuracy of ±5%. ^1H N.m.r. spectra of phenolic compounds were recorded on a Varian HA 100 or XL 100 spectrometer with hexadeuteriodimethyl sulphoxide as solvent and tetramethylsilane as internal reference. ^1H N.m.r. spectra of trimethylsilyl derivatives were recorded for solutions in carbon tetrachloride with dichloromethane as internal reference. Mass spectra were determined with an A.E.I. MS9 spectrometer with a direct insertion probe. Accurate mass measurements were made relative to fragmentations from heptacosafluorotri-*N*-butylamine at a resolving power of 15 000. Microanalyses were carried out by Mr. O. D. Hughes at Swansea. Tetracyclines were chromatographed on Whatman no. 1 paper lightly sprayed with McIlvaine buffer (pH 3.5) and developed in the system nitromethane–toluene–butan-1-ol–pyridine (20 : 10 : 5 : 3 v/v).[37] Thin-layer plates were prepared from Kieselgel G (Merck).

4-Dedimethylamino-12a-deoxyterramycin (47).—The method of Woodward *et al.*[38] was employed, with some modifications. Zinc dust (96.0 g, 1.47 mol) was added in portions to a stirred solution of terramycin dihydrate (95.6 g, 0.193 mol) in glacial acetic acid (620 ml) at 27 °C. The mixture was stirred at this temperature until paper chromatography indicated that terramycin (R_F 0.3) and the intermediates 4-*epi*-terramycin (R_F 0.15) and 4-dedimethylaminoterramycin (R_F 0.4) had been converted entirely into 4-dedimethylamino-12a-deoxyterramycin (R_F 0.95) (28 h). Zinc was removed by filtration and the filtrate evaporated at 35 °C to a viscous liquid. This residue was stirred with water (1.0 l) and the resulting amorphous solid filtered off, washed with water, dried, and extracted exhaustively (Soxhlet) with ether (2.0 l). The extract was concentrated and pentane was added to precipitate the product as a pale yellow solid (44.1 g, 57%) (Found: C, 60.0; H, 5.2; N, 3.25. Calc. for $C_{20}H_{19}NO_8$: C, 59.85; H, 4.8; N, 3.5%), M^+ 401, $\nu_{max.}$ 3 400, 2 980, 2 900, and 1 640 cm^{-1}, $\lambda_{max.}$ (MeOH–5M-HCl, 999 : 1) 217, 263, and 320 nm (log ε 4.22, 4.35, and 4.19).

6-Methylpretetramid (1).—A solution of 4-dedimethylamino-12a-deoxyterramycin (47) (40.0 g, 0.010 mol) in tetrahydrofuran (430 ml) and conc. HCl (200 ml) was refluxed for 2 h and then kept at 0 °C for 1 h. The solid was collected, washed with tetrahydrofuran (150 ml) and methanol (150 ml), and dried to give the product (1) as bright orange microcrystals (26.7 g, 73%), m.p. 270—300° (decomp.) (Found: C, 65.9; H, 4.0; N, 3.6. Calc. for $C_{20}H_{15}NO_6$: C, 65.75; H, 4.1; N, 3.8%), M^+ 365, $\nu_{max.}$ 3 440, 3 380, 3 200, 2 800—2 500, 1 660, 1 620, and 1 590 cm^{-1}, $\lambda_{max.}$ (conc. H_2SO_4–sat. aq. sodium tetraborate, 99 : 1) 236, 263, 278, 295sh, 327, 342, 398, and 510 nm (log ε 4.34, 4.45, 4.42, 4.35, 4.19, 4.19, 4.22, and 4.24).

Oxygenation of 6-Methylpretetramid in Dimethylformamide–Aqueous Alkali.[39]—A suspension of 6-methylpretetramid (1) (1.0 g, 2.74 mmol) in a mixture of dimethylformamide (125 ml) and 0.1M-potassium hydroxide (125 ml) was shaken in an oxygen burette. Oxygenation ceased after absorption of 55 ml (2.46 mmol) of oxygen during 50 min. The solution was diluted with phosphate buffer (0.1M-K_2HPO_4–0.1M-KH_2PO_4, 1 : 1; 125 ml), adjusted to pH 7.0 with 2M-HCl, and extracted with isobutyl methyl ketone (5 × 400 ml). The combined extracts were washed

[37] C. R. Stephens, J. J. Beereboom, H. H. Rennhard, P. N. Gordon, K. Murai. R. K. Blackwood, and M. D. von Wittenau, *J. Amer. Chem. Soc.*, 1963, **85**, 2043.

[38] F. A. Hochstein, C. R. Stephens, L. H. Conover, P. P. Regna, R. Pasternack, P. N. Gordon, F. J. Pilgrim, K. J. Brunnings, and R. B. Woodward, *J. Amer. Chem. Soc.*, 1953, **75**, 5455.

[39] T. E. Winters, Ph.D. Thesis, University of Wales, 1967.

with 0.01M-HCl (1.0 l) and water (2 × 1.0 l), dried ($MgSO_4$), and kept at 0 °C overnight to deposit a deep red solid. This was collected to yield a quinonoid byproduct (10 mg). The filtrate was concentrated and cooled to yield the crude product (0.53 g) as orange-red microcrystals. This material contained traces of two quinonoid byproducts which were removed by column chromatography. A solution of the oxygenation product (100 mg) in dimethylformamide (5 ml) was applied to a column of polyamide (Woelm; 55 × 2.5 cm i.d.) which was eluted with dimethylformamide. The major component was obtained as a bright yellow solution (500 ml) which was concentrated *in vacuo* to 2 ml and diluted with isobutyl methyl ketone (100 ml). The solution was washed with 0.01M-HCl (2 × 100 ml) and water (2 × 100 ml), dried, and concentrated to yield the pure *product* (29) (30 mg) as bright orange microcrystals, m.p. 195—220° (decomp.) (Found: C, 60.5; H, 3.6; N, 3.4. $C_{20}H_{15}NO_8$ requires C, 60.5; H, 3.8; N, 3.5%), $\nu_{max.}$ 3 480, 3 200, 1 670, 1 630, 1 620, and 1 600 cm^{-1}, $\lambda_{max.}$ (conc. H_2SO_4–sat. aq. sodium tetraborate, 99 : 1) 203, 278, 290sh, 345sh, 394sh, and 518 nm (log ε 4.35, 4.39, 4.38, 4.23, 4.02, and 4.11), *m/e* 397 (weak), 395, 393, 379, 376, 365, 362, 348, 336, 334, 333, 322, 320, 305, 292, 277, 264, 58, 57, 44, and 43, *m** 362, 346, 332, 318.5, 308, 294, 279.5, and 267 (Found: *m/e* 397.0796. $C_{20}H_{15}NO_8$ requires 397.0797. Found: *m/e* 395.0640. $C_{20}H_{13}NO_8$ requires 395.0641. Found: *m/e* 393.0485. $C_{20}H_{11}NO_8$ requires 393.0485. Found: *m/e* 379.0692. $C_{20}H_{13}NO_7$ requires 379.0692. Found: *m/e* 376.0217. $C_{20}H_8O_8$ requires 376.0219. Found: *m/e* 365.0897. $C_{20}H_{15}NO_6$ requires 365.0899. Found: *m/e* 365.0536. $C_{19}H_{11}NO_7$ requires 365.0535. Found: *m/e* 348.0639. $C_{20}H_{12}O_6$ requires 348.0634. Found: *m/e* 348.0268. $C_{19}H_8O_7$ requires 348.0270).

A second component of the oxygenation product was obtained as a dark red solution (95 ml). Work-up as for the major product yielded a dark red solid (1 mg), $\lambda_{max.}$ (conc. H_2SO_4–sat. aq. sodium tetraborate) 230, 298, 421, 520sh, 555, 595, 640, and 694 nm (log ε 4.11, 4.62, 3.92, 3.82, 4.00, 4.09, 3.97, and 4.01). The mass spectrum suggested that this material was a quinone, and included, in addition to a molecular ion at *m/e* 379, a peak at *m/e* 381 which could only reasonably be attributed to formation of the corresponding hydroquinone. This phenomenon has been observed in the mass spectra of a number of quinones.[39] High resolution mass spectrometry gave the values: *m/e* 381.0848 ($C_{20}H_{15}NO_7$ requires 381.0848) and 379.0692 ($C_{20}H_{13}NO_7$ requires 379.0692).

A third component was obtained as a deep purple solution (90 ml) which, on work-up as before, yielded a dark red solid (1 mg) identical with that deposited on cooling the isobutyl methyl ketone extracts of the crude oxygenation product, $\lambda_{max.}$ (conc. H_2SO_4–sat. aq. sodium tetraborate) 273, 294, 305sh, 548, 575sh, and 630sh nm (log ε 4.33, 4.37, 4.34, 3.98, 3.96, and 3.77). The mass spectrum indicated that this product was quinonoid and included peaks for the molecular ion (*m/e* 379) and for the corresponding hydroquinone (*m/e* 381). High resolution mass spectrometry confirmed that this product was isomeric with the other byproduct (Found: *m/e* 379.0690. $C_{20}H_{13}NO_7$ requires 379.0692).

Acetylation of Compound (29).—A suspension of compound (29) (110 mg, 0.28 mmol) in acetic anhydride (20 ml) containing a trace of pyridine was shaken (20 °C; 24 h) and then poured into cold water (200 ml). The mixture was shaken vigorously and the resulting yellow solution was extracted with ethyl acetate (100 ml); the extract was washed with 5% sodium hydrogen carbonate solution (2 × 200 ml) and water (200 ml), dried ($MgSO_4$), and evaporated. The residue was triturated with ether to yield a *triacetyl derivative* as a bright yellow solid (87 mg, 60%), m.p. 151—155° (Found: C, 59.5; H, 4.0; N, 2.6; O, 33.9; Ac 23.5. $C_{26}H_{21}NO_{11}$ requires C, 59.65; H, 4.0; N, 2.7; O, 33.6; Ac, 24.7%), M^+ 523, $\nu_{max.}$ 3 460, 3 350, 3 200, 2 980, 2 940, 1 780, 1 690, 1 630, and 1 605 cm^{-1}, $\lambda_{max.}$ (MeOH–5M-HCl, 999 : 1) 243, 261, 280sh, and 415 nm (log ε 4.41, 4.40, 4.32, and 3.78), τ −1.16br (1 H, s, OH), 1.60br (1 H, s, CONH), 2.0—2.5 (3 H, m), 2.7—3.1 (2 H, m), 7.67 (3 H, s, Ac), 7.72 (3 H, s, Ac), 7.84 (3 H, s, Ac), and 8.24 (3 H, s, CH_3).

Hydrolysis of the triacetyl derivative by refluxing (30 min) in methanol–M-sodium hydroxide yielded compound (29).

Trimethylsilylation of Compound (29),—Hexamethyldisilazane (1.0 ml) and chlorotrimethylsilane (1.0 ml) were added to a suspension of compound (29) (100 mg) in pyridine (10 ml) and the mixture was shaken at 25 °C for 3 h. Solvent and excess of reagents were removed under reduced pressure and the residue was taken up in carbon tetrachloride (5 ml). The resulting suspension was filtered and the filtrate evaporated. The residue was dissolved in carbon tetrachloride and the solution evaporated, and this process was repeated to remove traces of pyridine. Removal of solvent gave the *hexakistrimethylsilyl derivative* (30) as a dark red gum, M^+ 829, $\nu_{max.}$(CCl_4) 3 500, 3 420, 2 960, 2 900, 1680, 1 625, and 1 610 cm^{-1}, τ −5.9 (0.66 H, s, OH), 2.50 (1 H, t, *J* 6 Hz, H-8), 2.53 (1 H, d, *J* 6 Hz, H-7), 2.60 (0.33 H, s, H-4 or -5), 2.63 (0.66 H, s, H-4 or -5), 3.12 (0.66 H, d, *J* 6 Hz, H-9), 3.16 (0.33 H, d, *J* 6 Hz, H-9), 3.21 (0.66 H, s, H-4 or -5), 3.25 (0.33 H, s, H-4 or -5), 4.76 (0.33 H, s, CON*H*$SiMe_3$), 8.18 (2 H, s, CH_3), 8.25 (1 H, s, CH_3), and 9.5—9.7 (54 H, m, 6 $SiMe_3$).

Treatment of the derivative (30) with aqueous methanol containing a trace of dilute HCl yielded compound (29).

Reduction of Compound (29) *with Hydrogen Iodide.*—Compound (29) (25 mg, 0.06 mmol) was dissolved in warm phenol (5.0 g) and hydriodic acid (64%; 2.5 ml) was added. The mixture was refluxed for 7 min and then poured into water (150 ml). The resulting bright orange solid was collected, washed with water (4 × 50 ml) and methanol (2 × 50 ml), and dried to give 6-methylpretetramid (1) (15 mg, 65%).

Reaction of Compound (29) *with Alkaline Hydrogen Peroxide.*—A solution of compound (29) (135 mg, 0.34 mmol) in 0.1M-potassium hydroxide (100 ml) was stirred at 60 °C during the addition of six portions of hydrogen peroxide (30%; 10 ml each) at intervals of 10 min. The mixture was stirred at 60 °C for a further 1.5 h, then cooled, acidified with 2M-HCl, and extracted with ethyl acetate (3 × 100 ml). The combined extracts were washed with 5% sodium hydrogen carbonate solution (2 × 100 ml) and discarded. The combined aqueous solutions were acidified with 2M-HCl and extracted with ethyl acetate (3 × 100 ml). The combined extracts containing acidic material were dried ($MgSO_4$) and evaporated to give a yellow gum. Trituration with light petroleum (b.p. 60—80 °C) yielded the anthrone (31) (38 mg, 33%) as a bright yellow solid, identical (m.p., i.r., u.v.) with an authentic sample prepared by oxidative degradation of the quinone (16).[9,30]

Reaction of Compound (29) *with Alkali.*—A solution of compound (29) (12 mg, 0.03 mmol) in 0.1M-potassium

hydroxide (20 ml) was stirred at 60 °C for 4 h. The deep purple solution was cooled, diluted with phosphate buffer (0.1M-K_2HPO_4–0.1M-KH_2PO_4, 1 : 1; 10 ml), and the pH adjusted with 2M-HCl to 7.0. When the solution was extracted with isobutyl methyl ketone (20 ml) only a trace of coloured material entered the organic phase. The pH of the aqueous solution was adjusted to 4.5 and the solution was extracted with isobutyl methyl ketone (20 ml). The resulting bright orange extract was washed with water (2 × 10 ml), dried ($MgSO_4$), and evaporated to yield a dark red solid (8.1 mg, 68%), identical (m.p., i.r., u.v.) with an authentic sample of the quinone (16).

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