Tetracycline Studies. Part III.[1] Trimethylsilyl Derivatives of Tetracyclines

By C. H. Hassall* and G. J. Thomas, Department of Chemistry, University College of Swansea, Singleton Park, Swansea

Tetracyclines, and ring A models, are converted by trimethylchlorosilane and hexamethyldisilazane in pyridine into trimethylsilyl derivatives which dissolve readily in common solvents. This has facilitated the use of n.m.r. and i.r. spectra in elucidating the molecular structures of tetracyclines.

INSOLUBILITY in organic solvents, high m.p.s, and poor resolution in qualitative and preparative chromatography have made difficult separations of mixtures of tetracyclines and the characterisation of small quantities of individual compounds either by chemical or by physical methods. We have, therefore, investigated the trimethylsilyl derivatives of tetracyclines, and related compounds, in the expectation that these derivatives could facilitate the determination of the molecular structures of new members of this group.

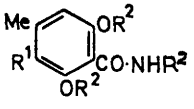
The results of these experiments are summarised in Table 1. Trimethylsilylation employed solutions or suspensions in pyridine containing chloro(trimethyl)-silane and hexamethyldisilazane; two reaction times, 1 min. and at least 12 hr., were employed for each compound. Each trimethylsilyl derivative was isolated, and the individuality of the volatile products was established by g.l.c. After its spectroscopic properties had been studied, each compound was hydrolysed; the hydrolysis product was identical with the starting material in each case. The number of trimethylsilyl groups in the derivatives was determined by integration of the n.m.r. signals in the region τ 9·5—10·2; the result was confirmed by the determination of the molecular weight through mass spectrometry.

Although benzamide has been trimethylsilylated,[2,3] it did not react under our conditions. In the case of salicylamide, *O*-trimethylsilylation alone took place. The structure of the product followed from the i.r. spectrum in carbon tetrachloride ($\nu_{max.}$ 1685 and 1570 cm.$^{-1}$, characteristic of an unassociated primary amide[4,5]). 2,6-Dihydroxy-4-methylbenzamide (I) and 3-acetyl-2,6-dihydroxy-4-methylbenzamide (III) gave tristrimethylsilyl derivatives (II) and (IV), but the 3-benzoyl analogue (V) formed only a bistrimethylsilyl derivative, even after extended reaction times. This has been assigned the structure (VI); the n.m.r. signal at τ −3·9 (1H) established that a chelated hydroxy-group was present, and the occurrence of an i.r. band at 1680 cm.$^{-1}$ indicated that the carbonyl of the 3-benzoyl group was not involved in intramolecular hydrogen bonding.

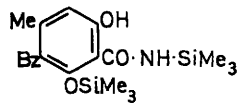
The six typical tetracyclines and tetracycline derivatives in Table 1 were trimethylsilylated readily. In four cases, the products obtained after treatment for *ca.* 1 min. at room temperature differed from those formed during 12 hr. Structures have been assigned to these derivatives largely in the light of their n.m.r. spectra. This has, in turn, established the importance of the derivatives in facilitating the use of n.m.r. spectrometry

[1] Part II, C. H. Hassall and G. Wootton, *J. Chem. Soc.* (*C*), 1969, 2439.
[2] P. L. De Benneville and M. J. Hurwitz, U.S.P. 2,876,209/1959 (*Chem. Abs.*, 1959, **53**, 12,321).
[3] J. Pump and U. Wannagat, *Monatsh.*, 1962, **93**, 352.
[4] Koji Nakanishi, 'Infrared Absorption Spectroscopy,' Holden-Day, San Francisco, 1962, p. 45.
[5] R. N. Kniseley, V. A. Fassel, E. L. Farquhar, and L. S. Gray, *Spectrochim. Acta*, 1962, **18**, 1217.

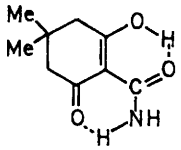
in tetracycline chemistry. As Wittenau and Blackwood [6] point out, the technique has been applied infrequently to this series owing to lack of suitable solvents. The



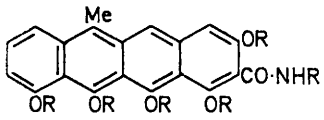
(I) $R^1 = H$, $R^2 = H$
(II) $R^1 = H$, $R^2 = Me_3Si$
(III) $R^1 = Ac$, $R^2 = H$
(IV) $R^1 = Ac$, $R^2 = Me_3Si$
(V) $R^1 = Bz$, $R^2 = H$



(VI)

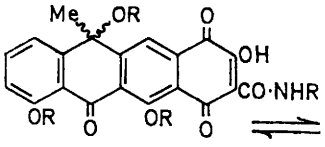


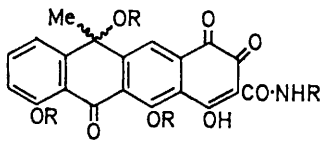
(VII)



(VIII) R=H (IX) R=Me_3Si

etc.





(XIII) R=H
(XIV) R=Me_3Si

(XV) R=H (XVI) R=Me_3Si

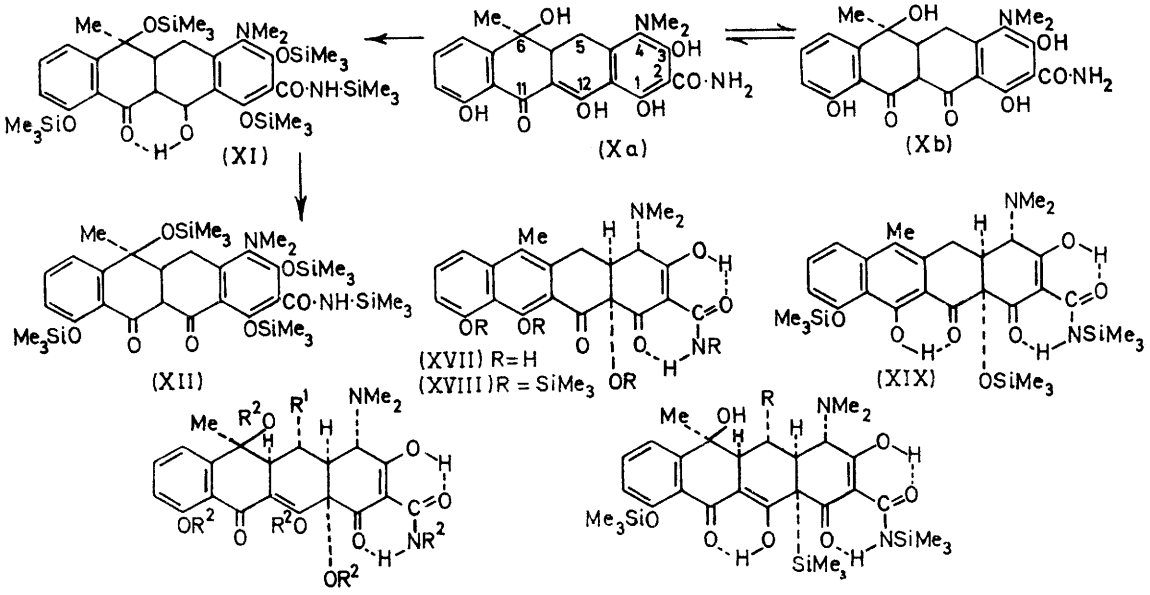
spectra of the majority of the compounds listed were determined for solutions in pyridine and trifluoroacetic acid, solvents that limit the scope of the study. They included data on the chemical shifts for 4-, 4a-, 5-, 5a-, and 6-protons, NMe_2, C-6 methyl, and C-6 methylene, but little information relating to other positions. In addition, Dudek and Volpp [7] have described the i.r. and n.m.r. characteristics of A-ring model carboxamides such as dimedone-2-carboxamide (VII), but prior to our study, related observations involving the carboxamide group of tetracyclines themselves have not been possible. The n.m.r. data in Table 2 were obtained with carbon tetrachloride as solvent and a Varian 100 MHz instrument. As the chemical shifts of protons of the trimethylsilyl groups are in the region τ 9·50—10·20, dichloromethane (τ 4·65) was employed as internal standard.

There is no ambiguity in the structure of hexakistrimethylsilyl-6-methylpretetramid (IX), but the case of 4a,12a-anhydrotetracycline is complicated by the existence of tautomeric forms such as (Xa) and (Xb). This accounts for the slow change in the u.v. spectrum of a solution in methanol. Trimethylsilylation for 1 min. and for 12 hr. gave pentakistrimethylsilyl derivatives in both cases. We attribute the structure (XI) to the first-formed product. The i.r. spectrum, unlike that of the later product had a broad band at 3150 cm.$^{-1}$ (OH chelated); it included, also, a band at 1680 cm.$^{-1}$, characteristic of the unchelated carboxamide system. The n.m.r. spectrum included a signal at τ —7·2 (1H) which we attribute to the strongly hydrogen-bonded proton of this formulation. Analogous cases have been observed;[8-10] the difference in the chemical shift of this function in the tetracycline derivative (XIV) (τ — 1·9) is attributed to weaker hydrogen bonding [7] due to less favourable stereochemistry. The spectra of both compounds included broad multiplets (total 3H) (C-5 and -5a) in the region τ 6·8—7·4 but, in addition, that of the second compound, formulated as (XII), included a broad band, τ 6·3—6·8 (1H). The proton at position 11a in the keto-form (XII) would be expected to give a signal at about τ 6·4.[11] Both tautomers, like hexakistrimethylsilyl-6-methylpretetramid (IX) and the model compounds (II) and (IV), had signals which we attribute to the unassociated proton of the carboxamide group, in the region τ 4·1—5·7. The n.m.r. spectrum of the product of trimethylsilylation of the quinone (XIII) also provided evidence of a mixture of tautomeric forms. The two signals in the region τ 8·28, 8·19 (3H), due to the 6-methyl protons, varied in intensity from sample to sample. Similarly, there was variation in the integration of signals in the region τ —8·0 to 0 due to hydrogen-bonded protons, and the individual peaks integrated for less than unity. The structures (XIV) and (XVI) have been assigned tentatively to constituents of the mixture of tetrakistrimethylsilyl derivatives.

There was no similar multiplicity in the n.m.r. spectra of the trimethylsilyl derivatives of the tetracyclines (XVII), (XX), and (XXII). Tristrimethylsilyl-5a,6-anhydrotetracycline is formulated as (XIX). The assignment of n.m.r. signals at τ 0·4, —4·4, and —7·0 to hydrogen-bonded NH, 11-OH, and 3-OH, respectively, can be related to similar chemical shifts in the carboxamide (VII) and in related ketones.[8] There was no band in the i.r. region above 3600 cm.$^{-1}$ (unassociated OH). Extended trimethylsilylation led to substitution of the 11-hydroxy-group and consequent loss of the n.m.r. signal at τ —4·4. For the tris- and tetrakis-trimethylsilyl derivatives of tetracycline (XX) and 5-hydroxytetracycline (XXII) respectively, we favour the structures (XXIV) and (XXV). The absence of any carbonyl i.r. absorption bands above 1660 cm.$^{-1}$ shows that all three carbonyl groups in these compounds are associated. This, together with the presence of an n.m.r. signal at τ —1·9, suggests that the 12-hydroxy-group has not been trimethylsilylated. The fact that this signal integrates for less than unity may be

[6] M. S. von Wittenau and R. K. Blackwood, *J. Org. Chem.*, 1966, **31**, 613.
[7] G. O. Dudek and G. P. Volpp, *J. Org. Chem.*, 1965, **30**, 50.
[8] G. O. Dudek and R. H. Holm, *J. Amer. Chem. Soc.*, 1961, **83**, 2099.
[9] S. Forsen and M. Nilsson, *Acta Chem. Scand.*, 1959, **13**, 1383.
[10] S. Forsen and M. Nilsson, *Acta Chem. Scand.*, 1960, **14**, 1333.
[11] R. M. Silverstein and G. C. Bassler, 'Spectrometric Identification of Organic Compounds,' Wiley, New York, 1963, p. 88.

due to the presence of some 12-keto-tautomers in concentrated solutions. Signals at τ 0·6 and −6·8 in tristrimethylsilyltetracycline (XXIV) and at τ 0·8 and −7·5 in tetrakistrimethylsilyl-5-hydroxytetracycline (XXV), provide evidence for the hydrogen-bonded carboxamide system; this resembles the case of 5a,6-anhydrotetracycline (XVII). By analogy, also, with 5a,6-anhydrotetracycline, and from the considerations of molecular models, it would be expected that the 12a-hydroxy-group would be easily silylated. The mass spectra of compounds (XXIV) and (XXV), unlike any



(XI) (Xa) (Xb) (XII) (XVII) R=H (XVIII) R = $SiMe_3$ (XIX)

(XX) R^1 = H, R^2 = H
(XXI) R^1 = $SiMe_3$, R^2 = H
(XXII) R^1 = H, R^2 = OH
(XXIII) R^1 = $SiMe_3$, R^2 = O·$SiMe_3$

(XXIV) R = H
(XXV) R = O·$SiMe_3$

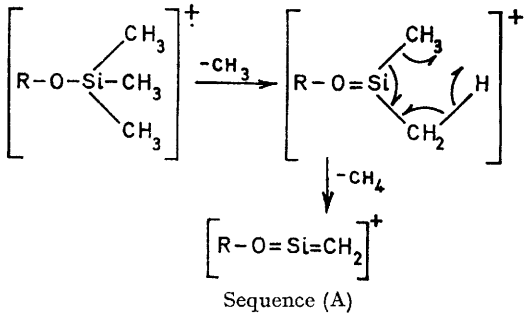
of the other derivatives studied, showed an initial loss of the elements of water. This we attribute to a dehydration process involving the 5a- and 6-positions. The structures (XXI) and (XXIII) for the corresponding pentakis- and hexakis-trimethylsilyl derivatives are supported by similar evidence (Tables 1 and 2). This investigation establishes that the trimethylsilylation of tetracyclines leads to the formation of derivatives that may be utilised for elucidating molecular structure by spectroscopic techniques. It deserves mention, also, that some of these trimethylsilyl derivatives may be employed for a convenient separation of tetracyclines by chromatography on polyamide columns. In a typical experiment, a mixture of hexatristrimethylsilyl-6-methylpretetramid (IX) and pentakistrimethylsilyltetracycline (XXI) in benzene was separated without significant loss, to give the pure constituents.

EXPERIMENTAL

M.p.s were determined with a Kofler hot-stage apparatus. I.r. spectra were recorded with Perkin-Elmer 257 and 457 spectrophotometers for solutions in carbon tetrachloride. ^{1}H N.m.r. spectra were recorded with Perkin-Elmer 60 MHz and Varian 100 MHz spectrometers.

Mass Spectra.—Mass spectra were determined with an A.E.I. MS9 double-focusing mass spectrometer. Less volatile samples were introduced at *ca.* 300° by a direct insertion probe, but the monobenzenoid derivatives were investigated by means of the gallium inlet system. An electron-beam energy of 70 ev was used. All ions having an abundance of 10% or more of the base peak are recorded. Ions of lesser abundance are recorded if they are of diagnostic value.

The parent ions of the trimethylsilylated tetracyclines were often of low intensity but large (M −15) peaks, arising from loss of a methyl group, were regularly observed. This loss was followed by a loss of a CH_4 fragment commonly associated with a metastable ion. We attribute this to the sequence (A). Abundant ions at m/e 147 $[Me_2Si{=}O{-}SiMe_3]^+$,



Sequence (A)

75 $[HO{=}SiMe_2]$,$^+$ 73 $[SiMe_3]^+$, and 45 $[H_2SiMe_2]^+$ were observed. The interpretation was complicated by the greater relative intensities of $(m + 1)/e$ and $(m + 2)/e$ ions than in

the case of common organic compounds free from silicon. This was due to relatively high abundance of the isotopes ^{29}Si (4·67%) and ^{30}Si (3·05%). The calculated [12] and observed relative intensities of m/e, $(m + 1)/e$ and $(m + 2)/e$ were in reasonable agreement. For example, in the case of the tetrakistrimethylsilyl quinone (XIV), the calculated values of $(M + 1)/M$ and $(M + 2)/M$ are 0·56 and 0·30 respectively; found: 0·7 ± 0·2 and 0·4 ± 0·2.

Chromatography.—Thin-layer plates were prepared from Kieselgel G (Merck) and developed with the systems (A) benzene–methanol–glacial acetic acid [10 : 2 : 1 (v/v)] and (B) ethyl acetate–glacial acetic acid [9 : 1 (v/v)]. Phenols were identified by spraying with ammonia and a freshly prepared solution of a stabilised diazonium salt of *o*-dianisidine (0·05 g.) in methanol–water [1 : 1 (v/v); 40 ml.]. Paper chromatography of tetracyclines employed the system of Stephens *et al.*[13]

Gas chromatography of the volatile trimethylsilyl derivatives employed F and M model 810 equipment with a hydrogen flame ionisation detector, stainless steel column

TABLE 1

Compound	Silylation procedure	τ Values for $SiMe_3$	Integral τ 9·5—10·2	M †	$\nu_{max.}$ * ($CO{\cdot}NH_2$)
1. Trimethylsilylsalicylamide	1 min./20° and 22 hr./80°	9·72, 9·66	9H	209	1685, 1565
2. Tristrimethylsilyl-2,6-dihydroxy-4-methylbenzamide (II)	1 min./20°	9·72, 9·70	27H	383	1675, obscured
3. Tristrimethylsilyl-3-acetyl-2,6-dihydroxy-4-methylbenzamide (IV)	1 min./20°	9·81, 9·69	27H	425	1670, 1565
4. Bistrimethylsilyl-3-benzoyl-2,6-dihydroxy-4-methylbenzamide (VI)	1 min./20° and 18 hr./90°	9·62, 9·53	18H	415	1660, 1560
5. Hexakistrimethylsilyl-6-methylpretetramid (IX)	12 hr./20° ‡	9·81, 9·70, 9·55	54H	797	1680, 1565sh
6. Tetrakistrimethylsilylquinone (XIV)	12 hr./20° ‡	9·94, 9·80, 9·66, 9·60, 9·50	36H	683	1690, 1575sh
7. Pentakistrimethylsilyl-4a,12a-anhydrotetracycline (XI)	1 min./20°	9·91, 9·85, 9·81, 9·77, 9·74, 9·69	45H	786	1680, 1550
8. Pentakistrimethylsilyl-4a,12a-anhydrotetracycline (XII)	12 hr./20° ‡	10·10, 9·82, 9·72, 9·68	45H	786	1680, 1550sh
9. Tristrimethylsilyl-5a,6-anhydrotetracycline (XIX)	1 min./20°	9·85, 9·63, 9·53	27H	642	1660, 1560sh
10. Tetrakistrimethylsilyl-5a,6-anhydrotetracycline (XVIII)	12 hr./20° ‡	9·96, 9·74, 9·71, 9·50	36H	714	1660, 1560
11. Tristrimethylsilyltetracycline (XXIV)	1 min./20°	9·80, 9·69, 9·57	27H	660	1660, obscured
12. Pentakistrimethylsilyltetracycline (XXI)	12 hr./20° ‡	10·06, 9·87, 9·71, 9·68, 9·55	45H	804	1660, 1565
13. Tetrakistrimethylsilyl-5-hydroxytetracycline (XXV)	1 min./20°	9·81, 9·75, 9·56	36H	748	1660, obscured
14. Hexakistrimethylsilyl-5-hydroxytetracycline (XXIII)	12 hr./20° ‡	9·72, 9·68, 9·53	54H	892	1660, 1540

* The bands were in accord with the assignments Amide I, Amide II, but in the latter case the assignment was less specific as there were additional bands in this region of the spectrum. † Determined by mass spectrometry. ‡ In these cases method (*b*) (see Experimental section) was employed; method (*a*) was used for all other preparations.

TABLE 2

Proton chemical shifts of trimethylsilylated tetracyclines and ring A models (in CCl_4)

Compound (see Table 1)	NH unassoc.	NH · · · O	3-OH	NMe_2	4-H	4a-H	5-H	5a-H	6-OH	6-Me	11-OH	12-OH	11-H
1. Trimethylsilylsalicylamide	3·4	2·0											
2. (II)	4·55												
3. (IV)	4·41												
4. (VI)	2·8	2·0	−3·9										
5. (IX)	5·75									7·33			
6. (XIV and tautomers)		−0·04	−4·3							8·28			
			−8·4										
		−1·94	−9·25							8·19			
7. (XI)	4·82			7·15			6·8	7·4		8·25		−7·2	
8. (XII)	4·81			7·10			6·8	7·4		8·15			6·3—6·8
9. (XIX)		0·45	−8·1	7·46	6·2—7·1					7·46	−4·4		
10. (XVIII)		0·47	−8·2	7·42	7·24	6·4—7·0				7·38			
11. (XXIV)		0·57	−6·8	7·40	6·5—7·8				5·10	8·25		−1·9	
12. (XXI)		0·60	−8·2	7·40	6·5—7·8					8·22			
13. (XXV)		0·79	−7·5	7·40	6·0—7·2				5·10	8·23		−1·9	
14. (XXIII)		0·78	−8·0	7·58	6·44		6·6—7·8			8·33			

[12] J. H. Beynon and A. E. Williams, 'Mass and Abundance Tables for Use in Mass Spectrometry,' Elsevier, London, 1963, p. viii.

[13] C. R. Stephens, J. R. Beereboom, H. H. Rennhard, P. N. Gordon, K. Murai, R. K. Blackwood, and M. S. von Wittenau, *J. Amer. Chem. Soc.*, 1963, **85**, 2647.

(9 ft. × 4 mm. i.d.) packed with 10% E30 on Diatoport W (60—80 mesh) (argon flow rate 60 ml./min.); linear temperature control programme:

$$\underset{\text{hold for 4 min.}}{180^\circ} \xrightarrow{60^\circ/\text{min.}} \underset{\text{hold}}{260^\circ}$$

with detector temperature 280° and injection port temperature 280°.

Silylation Method (*a*).—Hexamethyldisilazane (0·5 ml.) and chloro(trimethyl)silane (0·5 ml.) were added to the phenolic compound (50 mg.) in pyridine (3·0 ml.). A white precipitate was formed immediately. The mixture was set aside for 1 min., then solvent and excess of reagents were removed under reduced pressure. The solution obtained by treating the residue with carbon tetrachloride was filtered and evaporated to give a residue which was redissolved in carbon tetrachloride. This process was repeated until no pyridine could be detected in the residue. The trimethylsilyl derivative was then dissolved in carbon tetrachloride (0·5 ml.) for the determination of spectra.

Silylation Method (*b*).—Hexamethyldisilazane (1·0 ml.) and chloro(trimethyl)silane (1·0 ml.) were added to the phenolic compound (100 mg.) as a suspension or solution in pyridine (10·0 ml.). A white precipitate was formed. The mixture was shaken at room temperature for 12 hr. and worked up as in method (*a*).

In each case the product was an oil which was characterised by mass, n.m.r., and i.r. spectra (Tables 1 and 2). The trimethylsilyl derivative was converted back into starting material by treatment with 50% aqueous methanol [to which a trace of hydrochloric acid had been added as catalyst for all cases but the tetracyclines (XVII), (XX), and (XXII)].

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