then was kept at 0°. After the system reached the state of equilibrium the "living" polymers were "killed" by adding a few drops of water and M_e then was determined by the ultraviolet absorption of α-methylstyrene. The pertinent results are given in Table I.

TABLE I

SYSTEM α-METHYLSTYRENE–"LIVING" α-METHYLSTYRENE "TETRAMER." EQUILIBRIUM ESTABLISHED AT 0° IN TETRAHYDROFURAN; COUNTER-ION IS Na^+

Concn. "living" ends = E_0	M_0	M_0/E_0	M_e
	I Series		
0.068	0.0464	0.68	0.0346
.068	.0920	1.35	.0675
.068	.186	2.73	.135
.068	.281	4.13	.209
.068	.372	5.47	.304
.068	.465	6.84	.366
.068	.789	11 60	.543
	II Series		
0.0606	0.0275	0.45	0.0212
.0613	.0470	0.77	.0332
.0564	.0541	0.96	.0414
.0599	.0843	1.41	.0624
.0640	.137	2.14	.0995
.0655	.165	2.52	.114
.0599	.188	3.14	.134
.0617	.312	5.06	.212
.0607	.409	6.74	.302
.0592	.572	9.66	.428
.0613	.594	9.89	.434
.0584	1.115	19.09	.644
.0143	0.394	27.6	.372
.0164	0.985	61.6	.715

For a constant initial tetramer concentration the plot of M_e *versus* the initial monomer concentration M_0 is shown by Fig. 1. The experimental curve goes through the origin proving that the original "living" polymers were indeed *the* tetramers. These equilibria eventually are established

$$T^* + M \rightleftarrows P_5^* \qquad K_1$$

$$P_5^* + M \rightleftarrows P_6^* \qquad K_2$$

$$P_6^* + M \rightleftarrows P_7^* \qquad K_3, \text{ etc.}$$

and for each experiment listed in Table I K_1 may be calculated if it is assumed that $K_1 = K_2 = K_3 = \ldots = K_\infty$. The relation[6]

$$(M_0 - M_e)/E_0 = K_1 M_e/(1 - K_1 M_e)$$

is used in such calculations. The results, shown graphically in Fig. 2, demonstrate that the assumption of constant K's is unattainable. However, K_1 may be obtained by extrapolation to $M_0 = 0$ which leads to $K_1 = 4.9$ l./mole. Accepting this K_1 value and assuming now all the remaining K's equal to K_2 the apparent K_2 is calculated[7] as shown again in Fig. 2. The extrapolation gives $K_2 = 4.0$ l./mole. Similarly one calculates K_3 to be between 3.0-3.3 l./mole. For a large M_0 the plots of

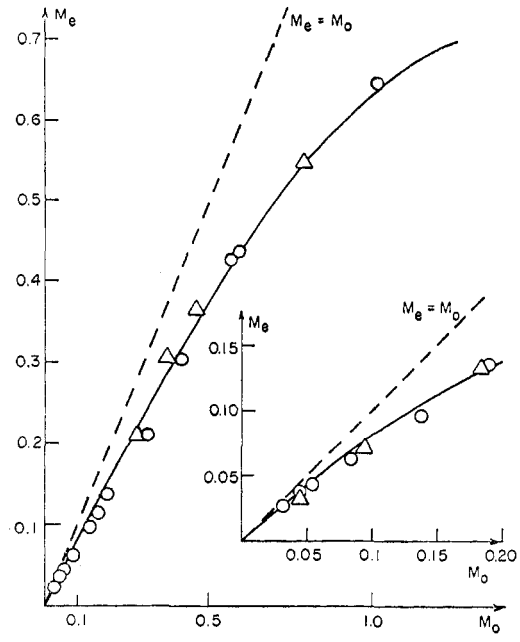

Fig. 1.

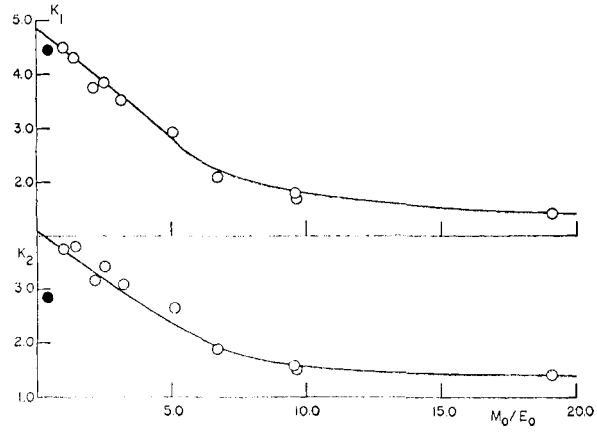

Fig. 2.

the apparent K_1's and K_2's approach asymptotically the same value of $K_\infty = 1.5$ l./mole, which agrees perfectly with those obtained from studies of equilibria involving high molecular weight polymers.[3,4]

The variations of K's value with n seems to indicate a dipole–dipole repulsion of the polymer ends. A good agreement is obtained between the observed and calculated K values if the C^-, Na^+ dipole moment is assumed to be 5 debyes.

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(6) A. V. Tobolsky, *J. Polymer Sci.*, **25**, 220 (1957); A. V. Tobolsky and A. Eisenberg, *J. Am. Chem. Soc.*, **81**, 780 (1959).

(7) K_1 is calculated from the equation $K_1 = M_e^{-1} - {}^1/_2K_1 \cdot \{\sqrt{1 + 4E_0/K_1Mi(M_0 - M_e)} - 1\}$.

6-METHYLENETETRACYCLINES.[1] I. A NEW CLASS OF TETRACYCLINE ANTIBIOTICS

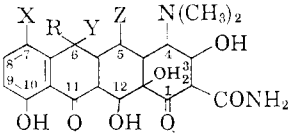
Sir:

Of special interest during recent years have been tetracycline antibiotics with structural modifica-

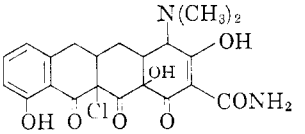
(1) Alternatively, the 6,13-anhydrotetracyclines, *cf.* expression XIV.

tions at the C.6 position.[2,3] We now wish to report a further major development in this area—preparation of 6-methylenetetracyclines.

In analogy with the fluorination studies described in the preceding communication,[4] two classes of 11a-substituted derivatives are obtained on chlorination of the tetracyclines. For example, treatment of 6-demethyl-6-deoxytetracycline (I)[3] with N-chlorosuccinimide in water yields *11a-chloro-6-demethyl-6-deoxytetracycline* (V), [m.p. 206° (dec.) $\lambda_{max}^{MeOH\cdot 0.01N\ HCl}$ 270 and 350 mμ; log ε 4.43 and 3.61

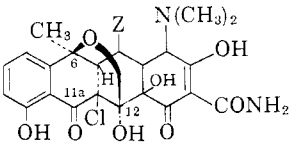


I, R = X = Y = Z = H
II, R = CH_3, X = Z = H, Y = OH
III, R = CH_3, X = H, Y = Z = OH
IV, R = CH_3, X = Cl, Y = OH, Z = H



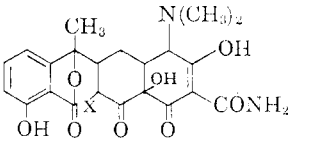
V

λ_{max}^{KBr} 5.71μ. *Anal.* Found for $C_{21}H_{21}N_2O_7Cl\cdot HNO_3$: C, 49.4; H, 4.3; N, 8.1; Cl, 6.7], a compound readily reconverted to starting material by catalytic hydrogenation, metal combination reduction, or even by mild sodium hydrosulfite reduction. In contrast, treatment of tetracycline (II) with N-chlorosuccinimide in 1,2-dimethoxyethane yields *11a-chlorotetracycline 6,12-hemiketal* (VI). [M.p.



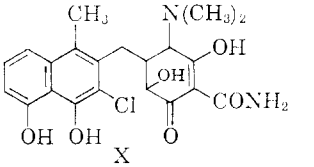
VI, Z = H
VII, Z = OH

194° (dec.) $\lambda_{max}^{MeOH\cdot 0.01N\ HCl}$ 258 and 343 mμ, log ε 4.33 and 3.60. No λ_{max}^{KBr} between 5 and 6μ. *Anal.* found for $C_{22}H_{24}N_2O_8Cl\cdot 0.5H_2O$: C, 54.0; H, 5.0; N, 5.8; Cl, 6.7.] The spectral properties of VI are very similar to those of 11a-fluorotetracycline 6,12-hemiketal.[4] Hydrosulfite reduction of VI regenerates tetracycline. In hot aqueous methanolic hydrochloric acid, VI does not undergo the classical tetracycline 5a,6-dehydration reaction,[4,8] but is converted to *11a-chloroisotetracycline* (VIII) [M.p. 229° (dec.) $\lambda^{MeOH\cdot 0.01N\ HCl}$ 240 and 272 mμ, log ε 4.11 and 4.19. λ_{max}^{KBr} 5.65 and 5.73μ. *Anal.* found for $C_{22}H_{23}N_2O_8Cl\cdot HCl$: C, 50.8; H, 4.8; N, 5.4; Cl, 13.6; Cl^-, 6.9], by an apparent hemiketal isomerization-cleavage sequence. Compound VIII is readily reduced to isotetracycline[5] (IX) with sodium hydrosulfite.



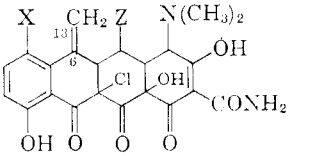
VIII, X = Cl
IX, X = H

On heating *in vacuo*, VI decomposes to a crude *1,8-dihydroxynaphthalene derivative* with probable structure X.



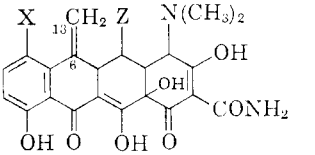
X

In anhydrous hydrogen fluoride, VI is dehydrated smoothly to *11a-chloro-6-methylenetetracycline* (XI).[6] [M.p. 209° (dec.) $\lambda_{max}^{MeOH\cdot 0.01N HCl}$ 237, 274 and 365 mμ, log ε 4.34, 4.27 and 3.60. λ_{max}^{KBr} 5.7μ *Anal.* found for $C_{22}H_{21}N_2O_7Cl\cdot HCl$: C, 53.1; H, 4.5; N, 5.7; Cl^-, 6.7.] Ozonolysis of XI in water generates formaldehyde.[7]



XI, X = Z = H
XII, X = H, Z = OH
XIII, X = Cl, Z = OH

Sodium hydrosulfite reduction of XI in water produces *6-methylenetetracycline* (XIV) [m.p. 213° (dec.) $\lambda_{max}^{MeOH\cdot 0.01N HCl}$ 254 and 353 mμ, log ε 4.35



XIV, X = Z = H
XV, X = H, Z = OH
XVI, X = Cl, Z = H
XVII, X = Cl, Z = OH

and 4.19. *Anal.* found for $C_{22}H_{22}N_2O_7\cdot HCl$: C, 57.1; H, 5.1; N, 6.1; Cl^-, 7.7]. On treatment with anhydrous hydrogen fluoride, XIV undergoes rapid rearrangement to 5a,6-anhydrotetracycline (XVIII).[8] Ozonolysis of XIV produces formaldehyde.[7]

By the same sequence (chlorination, dehydration and reduction), 5-hydroxytetracycline (III) is transformed into *6-methylene-5-hydroxytetra-*

(2) The 6-demethyltetracyclines: J. R. D. McCormick, N. O. Sjolander, V. Hirsch, E. R. Jensen and A. P. Doerschuk, *J. Am. Chem. Soc.*, **79**, 4561 (1957).

(3) The 6-deoxytetracyclines: C. R. Stephens, K. Murai, H. H. Rennhard, L. H. Conover and K. J. Brunings, *ibid.*, **80**, 5324 (1958).

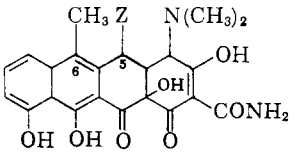
(4) H. H. Rennhard, R. K. Blackwood and C. R. Stephens, *ibid.*, **83**, 2775 (1961).

(5) J. H. Boothe, J. Morton, J. P. Petisi, R. G. Wilkinson and J. H. Williams, "Antibiotics Annual, 1953–1954," Medical Encyclopedia, Inc., New York, N. Y., 1953, p. 47.

(6) 11a-Fluorotetracycline 6,12-hemiketal (ref. 4) is also dehydrated to its *6-methylene derivative* by liquid hydrogen fluoride. 11a-Fluoro-6-demethyltetracycline 6,12-hemiketal is stable under similar conditions.

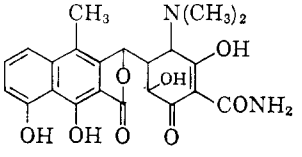
(7) Isolated in 19–23% yield as its dimedone adduct. Blank reactions on II and VI gave no significant quantity of formaldehyde. C-Methyl analyses on XI, XIV and XV, with appropriate blanks, also confirm the methylene structure.

(8) C. W. Waller, B. L. Hutchings, R. W. Broschard, A. A. Goldman, W. J. Stein, C. F. Wolf and J. H. Williams, *J. Am. Chem. Soc.*, **74**, 4981 (1952).



XVIII, Z = H
XIX, Z = OH

cycline (XV).[9] [M.p. 205° (dec.) $\lambda_{max}^{MeOH \cdot 0.01\ N\ HCl}$ 253 and 345 mμ log ε 4.37 and 4.19. *Anal.* found for $C_{22}H_{22}O_8N_2 \cdot HCl \cdot 0.5H_2O \cdot 0.5CH_3OH$: C, 54.0; H, 5.3; N, 5.4; Cl^-, 6.9; H_2O, 1.9; OCH_3, 3.1]. That XV possesses the methylene structure is deduced from spectral properties, from formation of formaldehyde on ozonolysis,[7] and from its conversion by treatment with hot aqueous acid to the apoterramycins (XX)[10] via the intermediate acid unstable (XIX) 5a,6-anhydro-5-hydroxytetracycline.[10]



XX

Chlorination of *11a-chloro-6-methylene-5-hydroxytetracycline* (XII) with N-chlorosuccinimide in liquid hydrogen fluoride, then hydrosulfite reduction of the intermediate *dichloro derivative* XIII, yields (XVII) *7-chloro-6-methylene-5-hydroxytetracycline* [$\lambda_{max}^{CH_3OH \cdot 0.01\ N\ HCl}$ 245, 347 mμ, log ε 4.34, 4.10. *Anal.* Found for $C_{22}H_{21}N_2O_8Cl$: C, 55.3; H, 4.4; N, 5.6; Cl, 7.5]. The assignment of structure to XVII is based on composition, spectral data, and the observation that an exhaustive methylation–oxidation sequence converts it to 6-chloro-3-methoxyphthalic anhydride.[11]

The 6-methylenetetracyclines (XIV, XV, XVI, XVII) described herein show broad *in vitro* antimicrobial activity.[12] Illustrative are the biological assay data shown in Table I.

TABLE I

Compound	Biological assay, 5-hydroxytetracycline units per mg.[13]
Tetracycline(II)	1000
5-Hydroxytetracycline(III)	1000
6-Methylenetetracycline(XIV)	1200
6-Methylene-5-hydroxytetracycline (XV)	2300
7-Chloro-6-methylene-5-hydroxytetracycline (XVII)	6300

(9) Similarly 7-chlorotetracycline (IV) has been converted to *7-chloro-6-methylenetetracycline* (XVI).

(10) Both the α and β forms of apoterramycin are obtained—*cf.* F. A. Hochstein, C. R. Stephens, L. H. Conover, P. P. Regna, R. Pasternack, K. J. Brunings and R. B. Woodward, *J. Am. Chem. Soc.*, **75**, 5455 (1953).

(11) S. Kushner, J. H. Boothe, J. Morton, J. Petisi and J. H. Williams, *ibid.*, **74**, 3710 (1952).

(12) We are indebted to Drs. A. R. English, T. J. McBride and B. A. Sobin for permission to disclose their unpublished biological studies.

(13) Based on the standard 5-hydroxytetracycline biological assay against *Klebsiella pneumoniae*, *cf.* R. C. Kersey, *J. Am. Pharm. Assoc.*, **39**, 252 (1950). We are indebted to Mr. J. J. Smith and his associates for these assays.

Of particular potential significance is 6-methylene-5-hydroxytetracycline (XV), a compound which shows evidence of superior therapeutic effect in animals as compared to earlier tetracyclines.[12]

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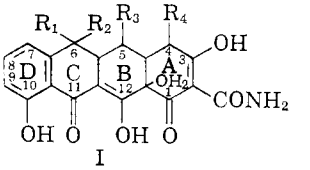
RECEIVED MAY 8, 1961

FLUOROTETRACYCLINES. I. PERCHLORYL FLUORIDE STUDIES IN THE TETRACYCLINE SERIES

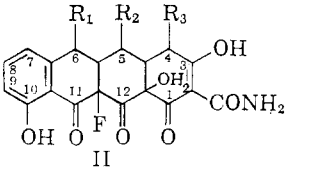
Sir:

Interaction of perchloryl fluoride[1] under suitable conditions[2] with a variety of tetracyclines (Ia-Ih) has resulted in two classes of active methylene fluorination products (II and III). These substances are of unusual interest as intermediates for further transformations and as model substances in more clearly defining various questions of stereochemistry and reaction mechanism in the tetracycline series.



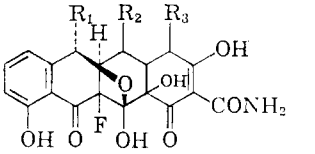
I

Ia, $R_1 = R_2 = R_3 = H$, $R_4 = NMe_2$
Ib, $R_1 = Me$, $R_2 = H$, $R_3 = OH$, $R_4 = NMe_2$
Ic, $R_1 = Me$, $R_2 = OH$, $R_3 = H$, $R_4 = NMe_2$
Id, $R_1 = Me$, $R_2 = R_3 = OH$, $R_4 = NMe_2$
Ie, $R_1 = R_3 = H$, $R_2 = OH$, $R_4 = NMe_2$
If, $R_1 = Me$, $R_2 = OH$, $R_3 = R_4 = H$
Ig, $R_1 = Me$, $R_2 = R_3 = OH$, $R_4 = H$
Ih, $R_1 = R_2 = R_3 = R_4 = H$



II

IIa, $R_1 = R_2 = H$, $R_3 = NMe_2$
IIb, $R_1 = Me$, $R_2 = OH$, $R_3 = NMe_2$
IIc, $R_1 = R_2 = R_3 = H$



III

IIIa, $R_1 = Me$, $R_2 = H$, $R_3 = NMe_2$
IIIb, $R_1 = Me$, $R_2 = OH$, $R_3 = NMe_2$
IIIc, $R_1 = R_2 = H$, $R_3 = NMe_2$
IIId, $R_1 = Me$, $R_2 = R_3 = H$
IIIe, $R_1 = Me$, $R_2 = OH$, $R_3 = H$

(1) *Cf.* C. E. Inmann, R. E. Oesterling and E. A. Tyczkowski, *J. Am. Chem. Soc.*, **80**, 6533 (1958).

(2) These include: (i) passage of the gas (excess) into a cold methanolic solution of the antibiotic and one equivalent of sodium methoxide; (ii) a similar procedure substituting water as the solvent and two equivalents of alkali. Procedure (i), when applied to basic compounds, results directly in a crystalline precipitate of the hydrochlorate salt of the fluorinated product.