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Studies of the Reversible Epimerization Occurring in the Tetracycline Family. The Preparation, Properties and Proof of Structure of Some 4-*epi*-Tetracyclines

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The four known members of the tetracycline family of broad-spectrum antibiotics (tetracycline, 7-chlorotetracycline, 7-bromotetracycline and 5-hydroxytetracycline) can undergo a reversible epimerization at carbon four to form a new series of compounds, the 4-*epi*-tetracyclines. For each of the four epimeric pairs, the equilibrium mixture in a variety of solvents is composed of approximately equal amounts of the tetracycline and the 4-*epi*-tetracycline. The 4-*epi*-tetracyclines have been isolated from the equilibrium mixtures, crystallized, and characterized. The epimerization occurs in the pH range of approximately two to six and is aided by the presence of buffers; it becomes unmeasurably slow under more strongly acidic conditions or more strongly basic conditions. The proof that the new materials are carbon four epimers of the tetracyclines depends on consideration of (1) the conditions permitting and preventing epimerization, (2) the reversible nature of the epimerization, (3) the nature of the ultraviolet spectral differences between epimeric pair members and (4) the nature of the chemical transformations required to convert both members of an epimeric parent pair to a single substance.

The tetracycline family (I) consists of a small group of closely related hydronaphthacenes that are of interest because of their broad-spectrum antibiotic activity. The known members of this family are tetracycline,<sup>1</sup> 7-chlorotetracycline,<sup>1</sup> 7-bromotetracycline and 5-hydroxytetracycline.<sup>2</sup> The reactions of these compounds under strongly acidic conditions and under strongly alkaline conditions have been studied extensively in connection with the determinations of their structures.<sup>3-5</sup> It recently has been found that these substances, and certain of their derivatives, undergo a reversible isomerization under pH conditions in the range of two to six.<sup>6</sup> This reversible isomerization has been shown to be an epimerization occurring at carbon four, giving rise to a new series of compounds, the 4-*epi*-tetracyclines (II).<sup>7,8</sup> These new substances have been called the quatrimycins.<sup>6</sup> This paper describes conditions permitting and preventing the epimerization, methods of preparation and characteristics of some 4-*epi*-tetracyclines and evidence for the conclusion that the reaction leading to the new compounds is a reversible epimerization at carbon four.

The epimerization occurs in a variety of solvent systems within the pH range of approximately two to six; its rate is increased by the presence of certain anions, such as phosphate, citrate or acetate. Saturated solutions of tetracyclines or 4-

*epi*-tetracyclines in glacial acetic acid (25°) or in 2:1 dimethylformamide-1 *M* aqueous phosphate (pH 3.5, 25°) are equilibrated within 24 hours. The mole fraction of 4-*epi*-tetracycline in a given equilibrium mixture varies from 0.4 to 0.6. Tables I and II present examples of conditions allowing epimerization. Epimerization rates are unmeasurably small in solutions more acidic than pH 2. For tetracycline in 0.03 *N* HCl, the epimerization rate is unmeasurably small and acid-catalyzed dehydration to anhydrotetracycline (III)<sup>9,10</sup> does not occur; tetracycline in such solutions is chemically stable over long periods of time. Further increases in acid strength bring about dehydration and aromatization to anhydrotetracycline; in 1.0 *N* HCl, 4-*epi*-tetracycline yields, without measurable isomerization, anhydro-4-*epi*-tetracycline (IV) (Table III, Fig. 1). Anhydro-4-*epi*-tetracycline is

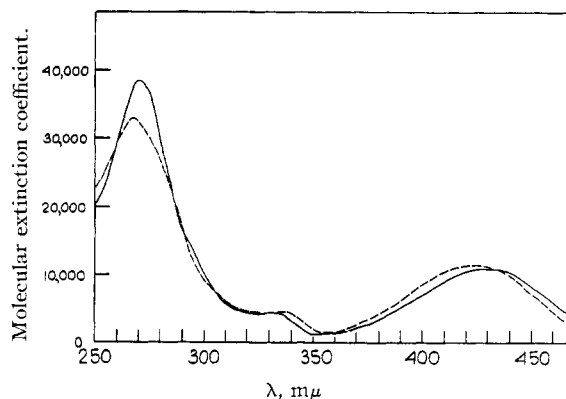


Fig. 1.—Ultraviolet absorption spectra in 0.1 *N* NaOH (aq.): — — —, anhydro-4-*epi*-tetracycline hydrochloride; —, anhydrotetracycline hydrochloride.

isomeric with, distinguishable from and reversibly convertible to anhydrotetracycline under conditions epimerizing the tetracyclines. Epimerization rates of the tetracyclines and their epimers are unmeasurably small in solutions more basic than

(1) The trademarks of the American Cyanamid Co. for tetracycline and 7-chlorotetracycline are Achromycin and Aureomycin, respectively.

(2) The trademarks of Chas. Pfizer and Co., Inc., for tetracycline and 5-hydroxytetracycline are Tetracycline and Terramycin, respectively.

(3) F. A. Hochstein, C. R. Stephens, L. H. Conover, P. P. Regna, R. Pasternack, K. J. Brunings and R. B. Woodward, *THIS JOURNAL*, **74**, 3708 (1952).

(4) C. R. Stephens, L. H. Conover, F. A. Hochstein, P. P. Regna, F. J. Pilgrim, K. J. Brunings and R. B. Woodward, *ibid.*, **74**, 4976 (1952).

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

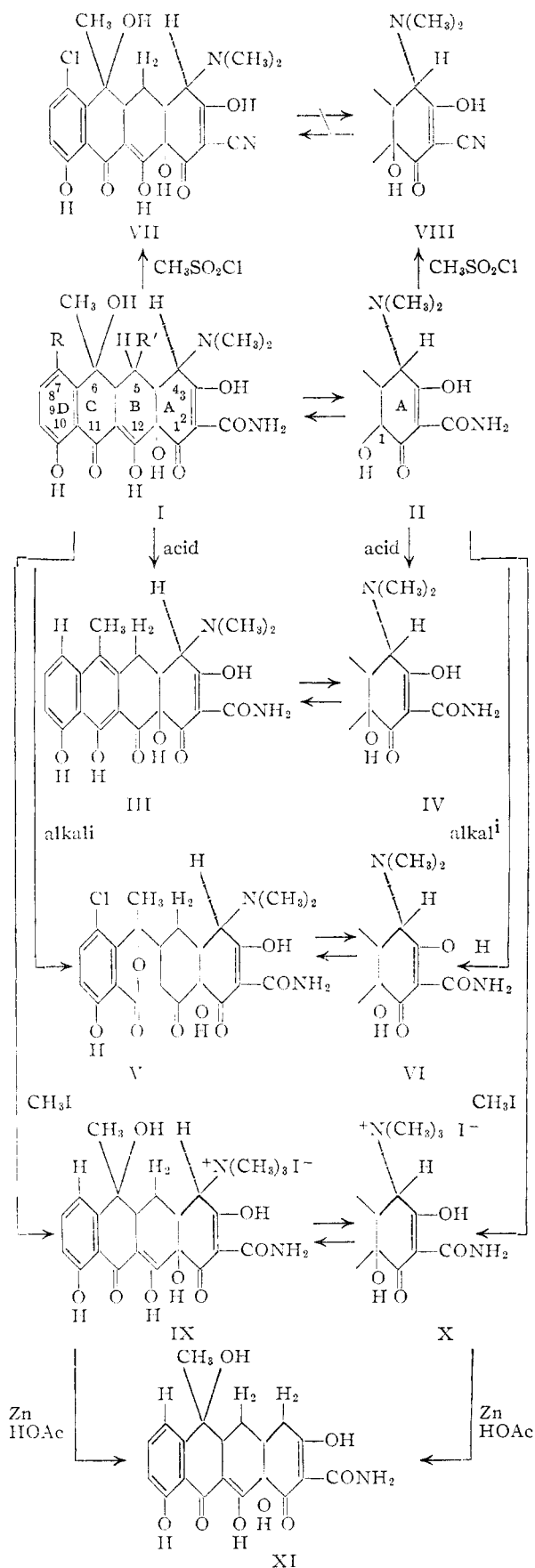
(6) A. P. Doerschuk, B. A. Bitler and J. R. D. McCormick, *ibid.*, **77**, 4687 (1955).

(7) C. R. Stephens, L. H. Conover, P. N. Gordon, F. C. Pennington, R. L. Wagner, K. J. Brunings and F. J. Pilgrim, *ibid.*, **78**, 1515 (1956).

(8) 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, *ibid.*, **78**, 3547 (1958). Although ref. 7 and 8 represent independent work, much of the discussion in ref. 8 and also in this paper applies to ref. 7.

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

(10) 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, *ibid.*, **76**, 3568 (1954).



	R	R'
Tetracycline	H	H
7-Chlorotetracycline	Cl	H
7-Bromotetracycline	Br	H
5-Hydroxytetracycline	H	OH

pH 9. In 0.1 *N* NaOH, 7-chloro-4-*epi*-tetracycline yields, without measurable epimerization, iso-7-chloro-4-*epi*-tetracycline (VI) (Table III, Fig. 2). Iso-7-chloro-4-*epi*-tetracycline is isomeric with, distinguishable from and reversibly convertible to iso-7-chlorotetracycline (V)<sup>10,11</sup> (Fig. 3) under conditions epimerizing the tetracyclines.

Separation of the 4-*epi*-tetracyclines from the equilibrium mixtures has been accomplished by partition chromatographic methods and also by differential crystallization methods convenient for the preparation of large quantities of materials. A tabulation of the properties of the 4-*epi*-tetracyclines and the corresponding tetracyclines is presented in Tables III and IV and Fig. 2. In general, the 4-*epi*-tetracycline member of each pair has the lesser ultraviolet absorbency in the 250–300  $m\mu$  region, the greater alkali stability, the greater acid stability, the more negative optical rotatory power, the greater water solubility, the greater tendency to be associated with the aqueous phase of partition separation systems and the lower *in vitro* antibacterial activity against *Staphylococcus aureus*. All these differences in properties have been used for analyzing mixtures of epimeric pair members. However, the most useful properties were the antibacterial activity against *S. aureus* and the shapes of the ultraviolet curves, expressed as ratios of the absorbencies at different wave lengths. Of the several absorbency ratios possible, the most useful for analytical purposes was the ratio between the absorbencies at 254 and at 267  $m\mu$ ; this method is called the Absorbency Ratio (AR) Assay. Where 220–300  $m\mu$  ultraviolet interference was present, use was made of the ratio of *S. aureus* antibacterial activity to ultraviolet absorbency at the long wave length ultraviolet absorption maximum ( $\sim 360 m\mu$ ); this method is called the Biological-Spectrophotometric Ratio (BSR) Assay. Ultraviolet interference in the 220–300  $m\mu$  region was more commonly encountered than was interference in the 360  $m\mu$  region, and the BSR assay was the more generally applicable of the two. Table V presents values of these properties for the pure compounds of the two series.

The constant nature throughout all pairs of the differences in properties between pair members, the fact that a single set of conditions can accomplish the epimerization of all members of the two series, and the fact that the limitations on the conditions allowing the epimerization are, insofar as they have been studied, similar for all members of the two series, indicate that the structural relationships between members of the pairs are the same for all pairs and that only a single process, common to all the cases, is involved in the conversions. Some confirmation of this comes from the fact that catalytic hydrogenation (palladium-on-carbon, 1.1 atmosphere) of 7-chloro-4-*epi*-tetracycline yields 4-*epi*-tetracycline.

(11) C. W. Waller, B. L. Hutchings, C. F. Wolf, A. A. Goldman, R. W. Broschard and J. H. Williams, *THIS JOURNAL*, **74**, 4981 (1952).

TABLE I

EXAMPLES OF CONDITIONS EPIMERIZING THE TETRACYCLINES, THE 4-*epi*-TETRACYCLINES AND SOME OF THEIR DERIVATIVES

Starting material concn., % (w./v.)	Solvent	Reaction time, hr.	Extent of epimerization, %
20% Tetracycline	2:1 MeOH/1 M aq. NaH <sub>2</sub> PO <sub>4</sub> , 25°, pH 3.5	4	47
20% Tetracycline	2:1 MeOH/1 M aq. NaH <sub>2</sub> PO <sub>4</sub> , 25°, pH 3.5	24	51 <sup>a</sup>
20% Tetracycline	2:1 MeOH/1 M aq. NaH <sub>2</sub> PO <sub>4</sub> , 25°, pH 3.5	72	51 <sup>a</sup>
20% Tetracycline	12:1 MeOH/4 M aq. NaH <sub>2</sub> PO <sub>4</sub> , pH 3.5	24	36
10% 4- <i>epi</i> -Tetracycline	1:0.05 H <sub>2</sub> O/88% HCOOH, 25°	5	45 <sup>a</sup>
10% 4- <i>epi</i> -Tetracycline	1:0.05 H <sub>2</sub> O/88% HCOOH, 43°	2	50 <sup>a</sup>
10% 4- <i>epi</i> -Tetracycline	1:0.05 H <sub>2</sub> O/88% HCOOH, 77°	0.083	60 <sup>a</sup>
10% Tetracycline	1:0.05 H <sub>2</sub> O/88% HCOOH, 77°	.083	33 <sup>a</sup>
5% Tetracycline	4.8% Zn(OAc) <sub>2</sub> dihydrate (4 equiv.) in gl. HOAc, 25°	.33	13
5% Tetracycline	4.8% Zn(OAc) <sub>2</sub> dihydrate (4 equiv.) in gl. HOAc, 25°	2.33	35
5% 4- <i>epi</i> -Tetracycline	4.8% Zn(OAc) <sub>2</sub> dihydrate (4 equiv.) in gl. HOAc, 25°	0.33	13
5% 4- <i>epi</i> -Tetracycline	4.8% Zn(OAc) <sub>2</sub> dihydrate (4 equiv.) in gl. HOAc, 25°	2.33	50
5% Tetracycline	4.8% Zn(OAc) <sub>2</sub> dihydrate (4 equiv.) in 1:1 HOAc-H <sub>2</sub> O, 25°	2.0	~3
5% Tetracycline	4.8% Zn(OAc) <sub>2</sub> dihydrate (4 equiv.) in 1:1 HOAc-H <sub>2</sub> O, 25°	4.0	~6
5% 4- <i>epi</i> -Tetracycline	4.8% Zn(OAc) <sub>2</sub> dihydrate (4 equiv.) in 1:1 HOAc-H <sub>2</sub> O, 25°	2.0	18
5% 4- <i>epi</i> -Tetracycline	4.8% Zn(OAc) <sub>2</sub> dihydrate (4 equiv.) in 1:1 HOAc-H <sub>2</sub> O, 25°	4.0	28
0.2 mg./ml. Tetracycline hydrochloride	Water, pH 3.5	48	7
14% 7-Chlorotetracycline	2:1 dimethylformamide/1 M aq. NaH <sub>2</sub> PO <sub>4</sub> , 25°, pH 3.5	24	44
0.4% 7-Chlorotetracycline	2:1 dimethylformamide/1 M aq. NaH <sub>2</sub> PO <sub>4</sub> , 25°	0.17	9
0.4% 7-Chlorotetracycline	2:1 dimethylformamide/1 M aq. NaH <sub>2</sub> PO <sub>4</sub> , 25°	0.50	17
0.4% 7-Chlorotetracycline	2:1 dimethylformamide/1 M aq. NaH <sub>2</sub> PO <sub>4</sub> , 25°	1.0	24
0.4% 7-Chlorotetracycline	2:1 dimethylformamide/1 M aq. NaH <sub>2</sub> PO <sub>4</sub> , 25°	3.0	35
0.4% 7-Chlorotetracycline	2:1 dimethylformamide/1 M aq. NaH <sub>2</sub> PO <sub>4</sub> , 25°	4.0	40 <sup>a</sup>
0.4% 7-Chlorotetracycline	2:1 dimethylformamide/1 M aq. NaH <sub>2</sub> PO <sub>4</sub> , 25°	24	41 <sup>a</sup>
1.2% 7-Chloro-4- <i>epi</i> -tetracycline ammonium salt	2:1 MeOH/1 M aq. NaH <sub>2</sub> PO <sub>4</sub> , 25°, pH 3.5	24	59 <sup>a</sup>
20% 7-Chlorotetracycline	Glacial acetic acid	3	32
12% 7-Bromotetracycline	2:1 dimethylformamide/1 M aq. NaH <sub>2</sub> PO <sub>4</sub> , 25°, pH 4.3	16	48
12% 7-Bromotetracycline	2:1 dimethylformamide/1 M aq. NaH <sub>2</sub> PO <sub>4</sub> , 25°, pH 4.3	24	51
7% 5-Hydroxytetracycline	1:1:1 tetrahydrofuran/dimethylformamide/1 M aq. NaH <sub>2</sub> PO <sub>4</sub> , 25°, pH 5.3	20	38
16% 5-Hydroxytetracycline	Glacial acetic acid, 25°	20	27
40 µg./ml. Iso-7-chlorotetracycline	2:1 dimethylformamide/1 M aq. NaH <sub>2</sub> PO <sub>4</sub> , 25°	0.083	12
40 µg./ml. Iso-7-chlorotetracycline	2:1 dimethylformamide/1 M aq. NaH <sub>2</sub> PO <sub>4</sub> , 25°	2.5	67 <sup>a</sup>
40 µg./ml. Iso-7-chlorotetracycline	2:1 dimethylformamide/1 M aq. NaH <sub>2</sub> PO <sub>4</sub> , 25°	3.5	68 <sup>a</sup>
40 µg./ml. Iso-7-chloro-4- <i>epi</i> -tetracycline	2:1 dimethylformamide/1 M aq. NaH <sub>2</sub> PO <sub>4</sub> , 25°	0.083	5
40 µg./ml. Iso-7-chloro-4- <i>epi</i> -tetracycline	2:1 dimethylformamide/1 M aq. NaH <sub>2</sub> PO <sub>4</sub> , 25°	2.0	32 <sup>a</sup>
40 µg./ml. Iso-7-chloro-4- <i>epi</i> -tetracycline	2:1 dimethylformamide/1 M aq. NaH <sub>2</sub> PO <sub>4</sub> , 25°	3.5	32 <sup>a</sup>
0.32% Anhydrotetracycline hydrochloride	2:1 MeOH/1 M aq. NaH <sub>2</sub> PO <sub>4</sub> , 25°	20	37 <sup>a</sup>
0.32% Anhydro-4- <i>epi</i> -tetracycline hydrochloride	2:1 MeOH/1 M aq. NaH <sub>2</sub> PO <sub>4</sub> , 25°	20	63 <sup>a</sup>
0.1% Benzenesulfonyl-4- <i>epi</i> -tetracyclonitrile	4:1 dimethylformamide/aq. H <sub>3</sub> PO <sub>4</sub> , 25°, pH 3.5	114	0
0.1% 7-Chloro-4- <i>epi</i> -tetracyclonitrile	4:1 dimethylformamide/aq. H <sub>3</sub> PO <sub>4</sub> , 40°, pH 1	44	0
0.1% 7-Chlorotetracyclonitrile	4:1 dimethylformamide/aq. H <sub>3</sub> PO <sub>4</sub> , 85°, pH 3.3	3	0
4.9% 4- <i>epi</i> -Tetracycline methiodide	2:1 dimethylformamide/1 M aq. NaH <sub>2</sub> PO <sub>4</sub> , 25°	16.5	65
5.5% Tetracycline methiodide	3.6% Zn(OAc) <sub>2</sub> in 1:1 HOAc-H <sub>2</sub> O, 25°	4.3	0
5.5% 4- <i>epi</i> -Tetracycline methiodide	3.6% Zn(OAc) <sub>2</sub> in 1:1 HOAc-H <sub>2</sub> O, 25°	4.3	~9

<sup>a</sup> The attainment of equilibrium was demonstrated by no further change with additional time.

TABLE II

EFFECT OF BUFFER CONCENTRATION ON THE EPIMERIZATION RATE OF A 20% TETRACYCLINE HYDROCHLORIDE SOLUTION IN 2:1 METHANOL/AQUEOUS BUFFER AT pH 4 AND 25°

Buffer concn.	Extent of epimerization, %				
	1 hr.	2 hr.	4 hr.	6 hr.	24 hr.
0.1 M NaH <sub>2</sub> PO <sub>4</sub>	4	12	35	40	49
1.0 M NaH <sub>2</sub> PO <sub>4</sub>	13	26	44	47	52

That the members of the pairs are not related as keto-enol tautomers is indicated by the facts that strong acid or strong base acts to prevent inter-conversion, rather than having a catalytic effect. The isomerization rate-pH value relationships and the effect of buffer anions suggest a mechanism of

interaction of both acid and base with the isoelectric form; an analogy may be drawn to the mutarotation of glucose.<sup>12</sup> It follows from the equilibrium mixture composition that the free energy change accompanying epimerization is small; therefore the structures of pair members are probably not greatly different. From the essential ultraviolet absorption similarity between pair members it follows that epimerization does not involve any extensive change in the chromophores. It is, however, significant that, in all cases, the pair members differ only slightly in ultraviolet absorption characteristics in the 340-380 mμ region

(12) L. P. Hammett, "Physical Organic Chemistry," McGraw-Hill Book Co., Inc., New York, N. Y., 1940, pp. 221, 338.

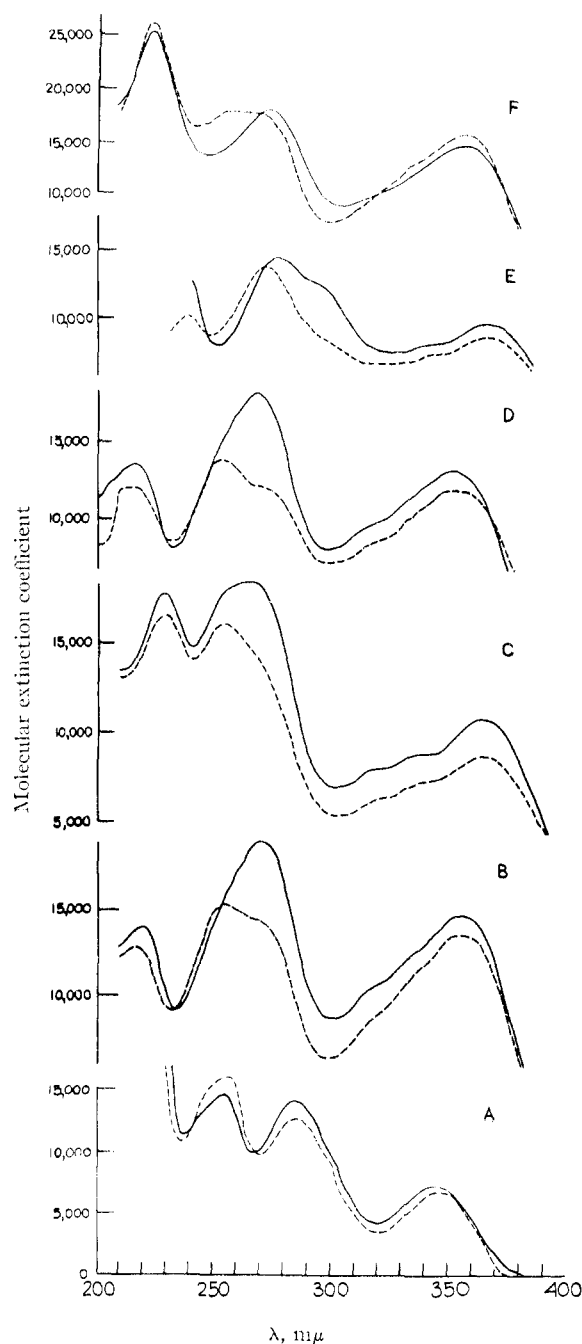


Fig. 2.—Ultraviolet absorption spectra: A, — — —, iso-7-chloro-4-*epi*-tetracycline; ———, iso-7-chlorotetracycline hydrochloride; both in 0.1 *N* NaOH (aq.); B, — — —, 4-*epi*-tetracycline ammonium salt monohydrate; ———, tetracycline hydrochloride; both in 0.1 *N* H<sub>2</sub>SO<sub>4</sub> (aq.); C, — — —, 7-chloro-4-*epi*-tetracycline ammonium salt monohydrate; ———, 7-chlorotetracycline hydrochloride; both in 0.1 *N* H<sub>2</sub>SO<sub>4</sub> (aq.) after 30 min.; D, — — —, 5-hydroxy-4-*epi*-tetracycline; ———, 5-hydroxytetracycline; both in 0.1 *N* H<sub>2</sub>SO<sub>4</sub> (aq.); E, — — —, 7-chloro-4-*epi*-tetracyclonitrile monohydrate; ———, 7-chlorotetracyclonitrile; both in 0.1 *N* H<sub>2</sub>SO<sub>4</sub> (aq.) containing 1% by volume of dimethylformamide; F, — — —, 4-*epi*-tetracycline methiodide; ———, tetracycline methiodide; both in 0.1 *N* H<sub>2</sub>SO<sub>4</sub> (aq.).

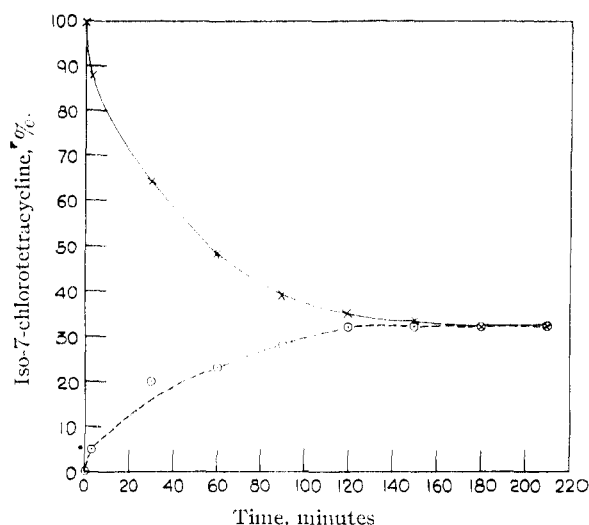


Figure 3.—The reversible epimerizations of (—○—○—) iso-7-chloro-4-*epi*-tetracycline and of (—x—x—x—) iso-7-chlorotetracycline in 2:1 dimethylformamide-1 *M* NaH<sub>2</sub>PO<sub>4</sub> (aq.) at 25°.

but substantially in the 250-300 mμ region, the difference being consistent in character and direction for all pairs. It has been shown that the

TABLE III

A COMPARISON OF THE PROPERTIES OF THE MEMBERS OF EPIMERIC PAIRS DERIVED FROM THE TETRACYCLINES<sup>a</sup> AND SOME OF THEIR DEGRADATION PRODUCTS

Compound	[α] <sub>D</sub> <sup>25</sup>	M.p., °C. (dec.)
4- <i>epi</i> -Tetracycline (II)	-348 <sup>cb</sup>	180-182
Tetracycline (I)	-235 <sup>b</sup>	162-164
7-Chloro-4- <i>epi</i> -tetracycline (II)	-350 <sup>b</sup>	209-213
7-Chlorotetracycline (I)	-245 <sup>b</sup>	168-169
5-Hydroxy-4- <i>epi</i> -tetracycline (II)	-253 <sup>b</sup>	163-164
5-Hydroxytetracycline (I)	-199 <sup>b</sup>	183-185
Anhydro-4- <i>epi</i> -tetracycline hydrochloride (IV)	-265 <sup>b</sup>	210-212
Anhydrotetracycline hydrochloride (III)	-210 <sup>b</sup>	204-206
Iso-7-chloro-4- <i>epi</i> -tetracycline monohydrate (VI)	-160 <sup>b</sup>	235-237
Iso-7-chlorotetracycline monohydrate (V)	-97 <sup>b</sup>	213-224
Benzenesulfonyl-4- <i>epi</i> -tetracyclonitrile monohydrate	-336 <sup>c</sup>	Dec. starting 200
Benzenesulfonyltetracyclonitrile dimethylformamide solvate	-116 <sup>c</sup>	Dec. starting 210
7-Chloro-4- <i>epi</i> -tetracyclonitrile monohydrate (VIII)	-300 <sup>c</sup>	Dec. starting 200
7-Chlorotetracyclonitrile (VII)	-338 <sup>c</sup>	Dec. starting 220
4- <i>epi</i> -Tetracycline methiodide (X)	-265 <sup>b</sup>	161-162
Tetracycline methiodide (IX)	-198 <sup>b</sup>	178-180
"Desdimethylamino-4- <i>epi</i> -tetracycline" (XI)	-251 <sup>d</sup>	195
Desdimethylaminotetracycline (XI)	-250 <sup>d</sup>	195

<sup>a</sup> Solutions of 7-bromotetracycline in solvents equilibrating the other tetracyclines changed in their ultraviolet absorption spectra and in their antibacterial activity toward *S. aureus* in directions and at rates completely comparable to those of the other tetracyclines, particularly to those of 7-chlorotetracycline. 7-Bromo-4-*epi*-tetracycline was isolated from these equilibrium mixtures as a crystalline solid having an ultraviolet absorption spectrum analogous to that of 7-chloro-4-*epi*-tetracycline and an antibacterial activity toward *S. aureus* of 5% that of 7-bromotetracycline. However, only small quantities were prepared from the available 7-bromotetracycline and recrystallized analytical material was not prepared. <sup>b</sup> Determined in 0.5% solution in 0.03 *N* HCl. <sup>c</sup> Determined in 0.5% solution in dimethylformamide. <sup>d</sup> Determined in 0.5% solution in Methyl Cellosolve.

TABLE IV  
ALKALINE AND ACID DESTRUCTION RATES OF THE MEMBERS OF EPIMERIC PAIRS DERIVED FROM THE TETRACYCLINES

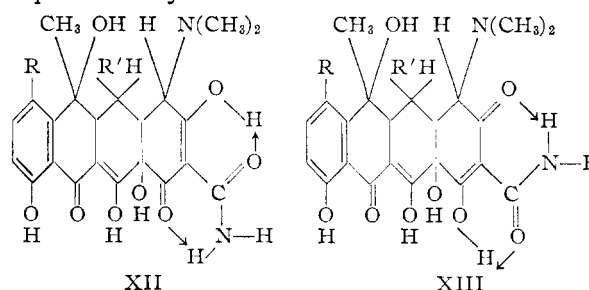
Compound	Half reaction times in alkali	Half reaction times in 1.0 N H <sub>2</sub> SO <sub>4</sub> (aq.) <sup>a</sup> , °C.	
4- <i>epi</i> -Tetracycline	225 min. in 0.1 N NaOH (aq.) at 60°	24.0	24
Tetracycline	101 min. in 0.1 N NaOH (aq.) at 60°	15.5	24
7-Chloro-4- <i>epi</i> tetracycline	154 min. in pH 8.8 carbonate buffer (aq.) at 29°	12.8	50
7-Chlorotetracycline	53 min. in pH 8.8 carbonate buffer (aq.) at 29°	7.3	50
5-Hydroxy-4- <i>epi</i> -tetracycline	21.8 hr. in 0.1 N NaOH (aq.) at 23°	6.0	50°
5-Hydroxytetracycline	10.9 hr. in 0.1 N NaOH (aq.) at 23°	6.3	50°

<sup>a</sup> For these cases, the product of the acid-catalyzed reaction is of the apoterramycin type<sup>13</sup> rather than of the anhydrotetracycline type.

tetracycline structure as a whole acts as two essentially independent chromophores.<sup>10</sup> The ring A chromophore contributes only to the 250–300 mμ absorption, while the rings B–C–D chromophore contributes to both the 250–300 mμ absorption and the 340–380 mμ absorption. The fact that epimerization changes principally the 250–300 mμ absorption indicates that a change in the ring A chromophore is involved. A partial confirmation of this is found in the fact that the anhydro compounds arising from the acid-catalyzed dehydration of tetracycline and of 4-*epi*-tetracycline retain the isomeric, distinguishable and reversibly interconvertible characteristics of the parent pair. Formation of these anhydro compounds involves aromatization of ring C, eliminating, as possible sites for

mild nature of the conditions allowing the change and the lack of side reactions.

The foregoing makes it most likely that the epimerization involves only a change in the configuration of C.4. The other possibility suggested by the published structures of the tetracyclines involves changes in the orientation of the 2-carboxamide group, whose free rotation might be prevented by strong hydrogen bonding with the two adjacent oxygen functions. The two interconvertible, isomeric possibilities resulting are represented by XII and XIII.



This relationship has been suggested earlier to explain the existence of the two isomeric, distinguishable and interconvertible apoterramycins.<sup>13</sup> However, the interconversion of the apoterramycins is catalyzed by either acid or base. An attempt to exclude the carboxamide orientation possibility took the form of dehydrating the non-symmetric carboxamide group to the linear nitrile group. Treatment of tetracycline and 4-*epi*-tetracycline with benzenesulfonyl chloride in pyridine<sup>13</sup> resulted in carboxamide dehydration accompanied by esterification to form benzenesulfonylnitriles.<sup>7,14</sup> The benzenesulfonyltetracyclinonitrile and the benzenesulfonyl-4-*epi*-tetracyclinonitrile were isomeric and distinguishable (Table III), but neither could be reversibly interconverted under conditions equilibrating the tetracyclines in four minutes nor under a variety of other conditions, including a series of pH values suggested by the gain in acid strength occurring on nitrile formation. To exclude the possibility that benzenesulfonyl substitution was preventing epimerization and the possibility that the two benzenesulfonylnitriles were distinguishable because of differences in position of benzenesulfonyl substitution, 7-chlorotetracycline and 7-chloro-4-*epi*-tetracycline were treated with methanesulfonyl chloride in pyridine to form the unsubstituted nitriles VII and VIII (Table III, Fig. 2).<sup>10,15</sup> The two unsubstituted nitriles were likewise isomeric and distinguishable, but could not be reversibly interconverted under the conditions described for the substituted nitriles. The experiment demonstrates that the intact carboxamide group, although it may be involved in the ready interconversion of the parent compounds, is not essential to the existence of isomeric pairs. Interconversion of the nitrile pair members under conditions equilibrating the tetracyclines, had it been possible as it was with other derivatives,

(14) The product of the corresponding reaction of 5-hydroxytetracycline has been described as being the 10-benzenesulfonylnitrile.<sup>13</sup>

(15) The method is that of R. Wilkinson, Research Division, American Cyanamid Company, who had previously prepared the nitrile of 7-chlorotetracycline by this method (unpublished).

TABLE V  
ULTRAVIOLET ABSORBENCY RATIOS AND BIOLOGICAL TO SPECTROPHOTOMETRIC ACTIVITY RATIOS USED FOR ASSAY OF EPIMERIC PAIR MIXTURES

Compound	Ratio of absorbency <sup>a</sup> at 254 mμ to absorbency at 287 mμ (AR)	Biological <sup>b</sup> to spectrophotometric activity <sup>a</sup> ratio (BSR) Value	Wave length used, mμ
Tetracycline hydrochloride	0.855	1.0	355
4- <i>epi</i> -Tetracycline hydrochloride	1.07	0.052	355
7-Chlorotetracycline hydrochloride	0.974	1.0	368
7-Chloro-4- <i>epi</i> -tetracycline hydrochloride	1.11	0.060	368
5-Hydroxytetracycline	0.836	1.0	352
5-Hydroxy-4- <i>epi</i> -tetracycline	1.13	0.032	352

<sup>a</sup> In 0.1 N H<sub>2</sub>SO<sub>4</sub>. <sup>b</sup> Turbidimetric assay against *S. aureus*.

the reversible change, all asymmetric carbon atoms except those of ring A. Inversion of configuration at C.4a or C.12a was ruled out because of the very

(13) 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, THIS JOURNAL, **75**, 5455 (1953).

would have provided a simple, certain proof that the nitriles were indeed related as were the parent compounds and therefore that the sulfonyl chloride reagents had acted in completely parallel ways on all four compounds. The lack of this proof, taken with the lack of any other independent proof of the relationship between the nitrile pair members, such as independent synthesis, weakens the argument that distinguishable sulfonyl chloride reaction products have eliminated the carboxamide orientation possibility and have proved the carbon four epimerization alternative.<sup>16</sup>

Further work to establish the fact of carbon four epimerization took the form of eliminating the asymmetry at carbon four by reductive removal of the 4-dimethylamino group. Zinc and glacial acetic acid at 30° for six hours accomplishes this.<sup>10,13</sup> However, preliminary experiments have shown that tetracycline and 4-*epi*-tetracycline are each essentially completely equilibrated in 2.5 hours in solution in glacial acetic acid-zinc acetate at 25°. Thus identical products could be obtained from both members of an epimeric pair by reduction under these conditions either through the elimination of the asymmetry at carbon four or through the epimerization to a common product during the reaction. The problem of distinguishing between these two alternatives was resolved by the finding that the methiodides could be reduced under non-epimerizing conditions. Tetracycline methiodide (IX) and 4-*epi*-tetracycline methiodide (X) (Table III, Fig. 2) were prepared<sup>17</sup> and found to be isomeric, distinguishable and reversibly interconvertible. Neither methiodide was measurably epimerized after remaining four hours in a simulated reduction solvent consisting of 1:1 acetic acid-water with four equivalents of zinc acetate. In 1:1 acetic acid-water, reduction with zinc for 30 minutes was sufficient for reductive cleavage of the trimethylammonium group from both methiodides. The two resulting desdimethylamino compounds were identical (XI, Table III). Criteria of identity included elemental analysis, optical rotation, mixed melting point, ultraviolet spectra, infrared spectra, counter-current distribution and solubility. This represents sufficient proof that the quatrmycins are the 4-*epi*-tetracyclines.

**Acknowledgment.**—The authors wish to acknowledge, with appreciation, the work of Neva-Tay Smith on the catalytic hydrogenation of 7-chloro-4-*epi*-tetracycline to 4-*epi*-tetracycline and the work of A. G. Mistretta on the counter-current distribution of desdimethylaminotetracycline. The

(16) A related experiment has shown that N-mono-*t*-butylanhydrotetracycline (J. E. Lynch and C. R. Stephens, "Antibiotics Annual," Medical Encyclopedia, Inc., 1955-1956, p. 466), when allowed to remain in solution in 2:1 dimethylformamide/1 *M* aqueous NaH<sub>2</sub>PO<sub>4</sub>, undergoes a change in ultraviolet absorption spectrum comparable in rate, direction and magnitude to that undergone by anhydrotetracycline under the same conditions. Although the experiment does not clarify the nature of the epimerization, it does provide an example of a change in the carboxamide group that does not interfere with easy epimerization. The N-mono-*t*-butylanhydrotetracycline was prepared by J. Boothe, Research Division, American Cyanamid Co.

(17) The methods of methiodide preparation and reduction and an authentic sample of tetracycline methiodide for comparison purposes were obtained from J. Boothe, Research Division, American Cyanamid Co.

antibacterial activity determinations and many of the instrumental assays, the elemental analyses and the optical rotation determinations were done under the direction of H. S. Kelsey, L. Brancone and W. Fulmor, respectively.

## Experimental

**4-*epi*-Tetracycline Ammonium Salt Monohydrate.**—A solution of 200 g. of tetracycline hydrochloride in 1 liter of 2:1 methanol-1 *M* aqueous NaH<sub>2</sub>PO<sub>4</sub> was allowed to remain 20 hr. at 25°; epimerization occurred to the extent of 58%, as judged by the AR assay. Acetone (1500 ml.) was added and the mixture allowed to remain for 2 hr. at 4°. The resulting precipitate (primarily inorganic salts) was filtered out and the filtrate concentrated *in vacuo* to 400 ml. Saturation of the solution with gaseous ammonia resulted in the formation of a crystalline product which was filtered out and washed twice with 100-ml. portions of concentrated ammonia water and twice with 200-ml. portions of acetone. After drying *in vacuo* the product weighed 166 g. and represented a recovery of 93% of the 4-*epi*-tetracycline formed by the epimerization, as judged by the AR assay. One kilogram of material prepared in this way (~70% 4-*epi*-tetracycline) was dissolved in 3 liters of water by adjusting the pH to 8 with 400 ml. of concentrated hydrochloric acid. After being clarified by filtration, the solution was adjusted to pH 8.8 with 100 ml. of concentrated ammonia water. Scratching induced the formation of a crystalline product which was filtered out, slurry-washed with one liter of an 80:15:5 mixture of acetone, water and concentrated ammonia water, and washed on the filter with two liters of acetone and then with one liter of acetone. After drying *in vacuo*, the product, a crystalline ammonium salt, weighed 373 g. and assayed 96% total tetracyclines and 95% 4-*epi*-tetracycline by the AR assay.<sup>18</sup> Reprocessing the product through the procedure described above yielded 213 g. of crystalline 4-*epi*-tetracycline ammonium salt monohydrate which assayed 100% 4-*epi*-tetracycline ammonium salt monohydrate by both the AR and BSR assays. The antibacterial activity (turbidimetric) against *S. aureus* was 6.4% that of tetracycline hydrochloride;  $[\alpha]_D^{25} -321^\circ$  (0.5% in 0.03 *N* HCl), m.p. 170° dec. The ultraviolet absorption spectrum in 0.1 *N* H<sub>2</sub>SO<sub>4</sub> (aq.) showed  $\lambda_{max}$  in m $\mu$  ( $\epsilon$ ): 216 (14,100), 255 (16,700), 270 (15,600, shoulder), 355 (14,800).

**Anal.** Calcd. for C<sub>22</sub>H<sub>27</sub>N<sub>3</sub>O<sub>8</sub>·H<sub>2</sub>O: C, 55.11; H, 6.11; N, 8.76; H<sub>2</sub>O, 3.76. Found: C, 55.68; H, 6.08; N, 8.82; H<sub>2</sub>O, 4.14.

**4-*epi*-Tetracycline Monohydrate.**—To a filtered solution of 390 g. of 4-*epi*-tetracycline ammonium salt in 2.5 liters of water and 60 ml. of concentrated hydrochloric acid (solution pH, 5.3) was added concentrated ammonia water to a final pH of 6.8. Scratching induced the formation of crystals which, after being washed twice with 1-liter portions of water and dried *in vacuo*, weighed 200 g. An analytical sample was prepared by dissolving 2 g. of this material in 25 ml. of water adjusted to pH 1.2 with concentrated hydrochloric acid, filtering, and adjusting the filtrate pH to 5.8 with concentrated ammonia water. The resulting crystalline product, after being washed with 10 ml. of water and 25 ml. of acetone and dried *in vacuo*, weighed 1.3 g. and assayed 100% 4-*epi*-tetracycline monohydrate by the AR and BSR assays. The antibacterial activity (turbidimetric) against *S. aureus* was 6.4% that of tetracycline hydrochloride;  $[\alpha]_D^{25} -335^\circ$  (0.5% in 0.03 *N* HCl), m.p. 178° dec. The ultraviolet absorption spectrum in 0.01 *N* H<sub>2</sub>SO<sub>4</sub> (aq.) showed  $\lambda_{max}$  in m $\mu$  ( $\epsilon$ ): 216 (13,900), 255 (16,400), 270 (15,200, shoulder), 355 (14,700).

**Anal.** Calcd. for C<sub>22</sub>H<sub>24</sub>N<sub>2</sub>O<sub>8</sub>·H<sub>2</sub>O: C, 57.12; H, 5.68; N, 6.06; H<sub>2</sub>O, 3.89. Found: C, 57.41; H, 5.62; N, 6.00; H<sub>2</sub>O, 3.90.

(18) Diluting the mother liquor with an equal volume of acetone and adjusting the pH to 9.0 with concentrated ammonia water yielded a second crop of crystals which, after washing and drying as before, weighed 444 g. and assayed 96% total tetracyclines and 70% 4-*epi*-tetracycline by the AR assay. The operations of this preparative process bring about little change in tetracycline or 4-*epi*-tetracycline except epimerization, so that recycle of second crystal crops, solvent-free mother liquors, etc., through re-equilibration and re-isolation is a feasible method of increasing the over-all yield.

**Tetracycline from 4-*epi*-Tetracycline.**—A solution of 5 g. of 4-*epi*-tetracycline ammonium salt monohydrate in 100 ml. of glacial acetic acid was allowed to remain 24 hr. at 25° and was then mixed with 400 ml. of water and seeded with a small quantity of tetracycline neutral. The crystals which formed on aging were collected, washed, dried and dissolved in 5 volumes of 4:1 Ethyl Cellosolve-*n*-butyl alcohol. The pH of the solution was adjusted to 1.5 with concentrated hydrochloric acid. The crystals which formed on aging were collected, washed, dried and identified as tetracycline hydrochloride (with possible trace amounts of 4-*epi*-tetracycline hydrochloride) by ultraviolet and infrared spectra, antibacterial activity (turbidimetric) against *S. aureus* and paper chromatography.

**7-Chloro-4-*epi*-tetracycline Ammonium Salt Monohydrate.**—A solution of 100 g. of 7-chlorotetracycline in 500 ml. of 2:1 dimethylformamide-1 *M* aqueous  $\text{NaH}_2\text{PO}_4$  was allowed to remain 20 hr. at 25°; epimerization occurred to the extent of 45%, as judged by the AR assay. Acetone (1 liter) was added and the resulting precipitate (primarily inorganic salts) filtered out. Adjustment of the filtrate to pH 8 with concentrated ammonia water yielded a crystalline product which, after being washed once with 25 ml. of a 2:1 acetone-dimethylformamide mixture and twice with 100-ml. portions of acetone and dried *in vacuo*, weighed 53.1 g. and represented a recovery of 78% of the 7-chloro-4-*epi*-tetracycline formed by the epimerization. To a filtered solution of 10 g. of this material in 25 ml. of water was added sufficient concentrated ammonia water to give a pH of 9.5. Aging for one hour yielded a crystalline product which, after being washed once with 10 ml. of 4:1 acetone-water and once with 20 ml. of acetone and dried *in vacuo*, weighed 4.2 g. and assayed 96% 7-chloro-4-*epi*-tetracycline ammonium salt by BSR assay. An analytical sample was prepared by dissolving 1.0 g. of this material in 10 ml. of water, filtering, adding 10 ml. of acetone to the filtrate, and adjusting the pH to 9.0 with concentrated ammonia water. The resulting crystalline product, after being washed with 3 ml. of 4:1 acetone-water and 10 ml. of acetone and dried *in vacuo*, weighed 0.64 g. and assayed 100% 7-chloro-4-*epi*-tetracycline ammonium salt monohydrate as judged by the AR and BSR assays. The antibacterial activity (turbidimetric) against *S. aureus* was 4.2% that of 7-chlorotetracycline hydrochloride;  $[\alpha]^{25}_D -321^\circ$  (0.5% in 0.03 *N* HCl), m.p. 163–164° dec. The ultraviolet absorption spectrum in 0.1 *N*  $\text{H}_2\text{SO}_4$  (aq.) showed  $\lambda_{\text{max}}$  in  $m\mu$  ( $\epsilon$ ): 230 (18,300), 255 (17,700), 368 (9,400).

*Anal.* Calcd. for  $\text{C}_{22}\text{H}_{26}\text{N}_2\text{ClO}_5 \cdot \text{H}_2\text{O}$ : C, 51.40; H, 5.49; N, 8.18; Cl, 6.90;  $\text{H}_2\text{O}$ , 3.50. Found: C, 51.88; H, 5.63; N, 8.58; Cl, 7.02;  $\text{H}_2\text{O}$ , 2.88.

**7-Chloro-4-*epi*-tetracycline.**—To a filtered solution of 49.7 g. of 7-chloro-4-*epi*-tetracycline ammonium salt monohydrate in 400 ml. of water was added 9.2 ml. of concentrated hydrochloric acid. The resulting crystalline product, after being washed with water and with acetone and dried *in vacuo*, weighed 22.7 g. An analytical sample was prepared by dissolving 1 g. of this material in 30 ml. of methanol and filtering. On standing, crystals appeared which, after being washed with water and with methanol and dried *in vacuo*, weighed 0.64 g. and assayed 100% 7-chloro-4-*epi*-tetracycline by AR and BSR assay. The antibacterial activity (turbidimetric) against *S. aureus* was 4.0% that of 7-chlorotetracycline hydrochloride;  $[\alpha]^{25}_D -350^\circ$  (0.5% in 0.03 *N* HCl), m.p. 209–213° dec. The ultraviolet absorption spectrum in 0.1 *N*  $\text{H}_2\text{SO}_4$  (aq.) showed  $\lambda_{\text{max}}$  in  $m\mu$  ( $\epsilon$ ): 230 (18,400), 255 (17,700), 368 (9,300).

*Anal.* Calcd. for  $\text{C}_{22}\text{H}_{28}\text{N}_2\text{ClO}_5$ : C, 55.18; H, 4.84; N, 5.84; Cl, 7.41. Found: C, 55.03; H, 4.90; N, 5.83; Cl, 7.36.

**7-Chloro-4-*epi*-tetracycline Hydrochloride.**—A solution of 40 g. of 7-chloro-4-*epi*-tetracycline ammonium salt monohydrate in 400 ml. of water was adjusted to pH 1 with 20 ml. of concentrated hydrochloric acid and aged for 30 min. The resulting crystalline product, after being washed with 25 ml. of cold water and dried *in vacuo*, weighed 19.3 g. and assayed 100% 7-chloro-4-*epi*-tetracycline hydrochloride by the AR and BSR assays. An analytical sample was prepared by dissolving 2.0 g. of this material in 75 ml. of 2-propanol, filtering, and aging. The resulting crystalline product, after being washed with 5 ml. of 2-propanol and dried *in vacuo*, weighed 1.7 g. and assayed 100% 7-chloro-4-*epi*-tetracycline hydrochloride by AR and BSR assay.

The antibacterial activity (turbidimetric) against *S. aureus* was 4.7% that of 7-chlorotetracycline hydrochloride;  $[\alpha]^{25}_D -312^\circ$  (0.5% in 0.03 *N* HCl), m.p. 211–214° dec. The ultraviolet absorption spectrum in 0.1 *N*  $\text{H}_2\text{SO}_4$  (aq.) showed  $\lambda_{\text{max}}$  in  $m\mu$  ( $\epsilon$ ): 230 (17,900), 255 (17,400), 368 (9,300).

*Anal.* Calcd. for  $\text{C}_{22}\text{H}_{24}\text{N}_2\text{Cl}_2\text{O}_5$ : C, 51.30; H, 4.69; N, 5.44; Cl, 13.77. Found: C, 51.80; H, 4.90; N, 5.26; Cl, 13.45.

**5-Hydroxy-4-*epi*-tetracycline.**—A solution of 100 g. of 5-hydroxytetracycline in 500 ml. of glacial acetic acid was allowed to remain 21 hr. at 25°; epimerization occurred to the extent of 29% as judged by the AR assay. Vacuum distillation to a volume of 200 ml., followed by the addition of 1500 ml. of water and vacuum distillation to 250 ml., resulted in the precipitation of unepimerized 5-hydroxytetracycline which, after being removed by filtration and dried *in vacuo*, weighed 61.7 g. and assayed 95% 5-hydroxytetracycline by microbiological assay (*S. aureus*) and 0% 5-hydroxy-4-*epi*-tetracycline by AR and BSR assay. The filtrate was adjusted to pH 7.7 with 70 ml. of concentrated ammonia water. The resulting crystalline product, after being washed twice with 50-ml. portions of water and dried *in vacuo*, weighed 14.9 g. and assayed 96% 5-hydroxy-4-*epi*-tetracycline by BSR assay. A filtered solution of 16.0 g. of material prepared in this way in 300 ml. of methanol and 1.5 ml. of concentrated hydrochloric acid was adjusted with concentrated ammonia water to pH 7 and aged for 2 hr. The resulting crystalline product was washed twice with 250-ml. portions of methanol, three times with 60-ml. portions of water, and twice with 50-ml. portions of acetone. After drying *in vacuo*, the product weighed 7.2 g. and assayed 100% 5-hydroxy-4-*epi*-tetracycline by AR assay and 98% by BSR assay. The antibacterial activity (turbidimetric) against *S. aureus* was 4.5% that of 5-hydroxytetracycline;  $[\alpha]^{25}_D -253^\circ$  (0.5% in 0.03 *N* HCl), m.p. 163–164° dec. The ultraviolet absorption spectrum in 0.1 *N*  $\text{H}_2\text{SO}_4$  (aq.) showed  $\lambda_{\text{max}}$  in  $m\mu$  ( $\epsilon$ ): 215 (12,200), 253 (14,200), 275 (12,000), 355 (12,100).

*Anal.* Calcd. for  $\text{C}_{22}\text{H}_{24}\text{N}_2\text{O}_6$ : C, 57.34; H, 5.30; N, 6.08. Found: C, 57.68; H, 5.34; N, 6.42.

**Anhydro-4-*epi*-tetracycline Hydrochloride.**—A solution of 10 g. of 4-*epi*-tetracycline in a mixture of 75 ml. of butanol, 19 ml. of water and 31 ml. of concentrated hydrochloric acid was heated to 70° for one hour, cooled and diluted with 40 ml. of water. The pH was adjusted to 5.5 with 5 *N* sodium hydroxide and the resulting precipitate washed with water and dried (6.5 g.). One gram of this precipitate was slurried in 30 ml. of methanol containing 1 ml. of concentrated hydrochloric acid and the mixture filtered. One ml. of concentrated hydrochloric acid was added to the filtrate. The crystalline product that resulted after two hours aging was washed with cold methanol and cold water and dried (0.45 g.);  $[\alpha]^{25}_D -265^\circ$  (0.5% in 0.03 *N* HCl), m.p. 210–212 dec. The ultraviolet absorption spectrum in 0.1 *N* NaOH (aq.) showed  $\lambda_{\text{max}}$  in  $m\mu$  ( $\epsilon$ ): 225 (22,200), 268 (29,600), 320–340 (4,200, shoulder), 425 (9,300).

*Anal.* Calcd. for  $\text{C}_{22}\text{H}_{28}\text{N}_2\text{ClO}_7$ : C, 57.08; H, 5.01; N, 6.05; Cl, 7.66. Found: C, 56.84; H, 5.34; N, 5.86; Cl, 7.59.

**Reversible Epimerization of Anhydrotetracycline Hydrochloride and Anhydro-4-*epi*-tetracycline Hydrochloride.**—A solution of 0.032 g. of anhydrotetracycline hydrochloride<sup>9,10</sup> in 10 ml. of 2:1 methanol/1 *M* aqueous  $\text{NaH}_2\text{PO}_4$  was allowed to remain at 25° for 20 hr. The same operation was carried out starting with anhydro-4-*epi*-tetracycline hydrochloride. The ultraviolet absorption spectra in 0.1 *N* sodium hydroxide were determined for both solutions before and after equilibration. At the end of 20 hr. the two solutions had identical ultraviolet absorption spectra which did not change further on additional standing. The ultraviolet absorption spectra of the equilibrium solutions corresponded to an equilibrium composition of 63% anhydrotetracycline and 37% anhydro-*epi*-tetracycline. The experiment demonstrates the approach to the same equilibrium composition starting from each of the two pure components.

**Iso-7-chloro-4-*epi*-tetracycline Monohydrate.**—A solution of 20 g. of 7-chloro-4-*epi*-tetracycline ammonium salt in 180 ml. of 0.1 *N* aqueous sodium hydroxide (pH of solution was



9.4) was allowed to remain 24 hr. at 25°. The ultraviolet absorption spectrum of the solution indicated that the rearrangement to iso-7-chloro-4-*epi*-tetracycline had occurred without epimerization and in 86% yield. Adjustment of the pH of the solution to 7.0 yielded a gelatinous precipitate which, after being slurry-washed with water and dried over phosphorus pentoxide *in vacuo*, weighed 12.4 g. (64% yield). The ultraviolet absorption spectrum of this crude iso-7-chloro-4-*epi*-tetracycline indicated that epimerization had occurred during work-up to the extent of about 30%. The contaminating iso-7-chlorotetracycline was removed by successive extractions with hot methanol to yield crystalline iso-7-chloro-4-*epi*-tetracycline monohydrate,  $[\alpha]^{25}_D -160^\circ$  (0.5% in 0.03 *N* HCl), m.p. 235–237° dec. The ultraviolet absorption spectrum in 0.1 *N* sodium hydroxide (aq.) showed  $\lambda_{max}$  in  $m\mu$  ( $\epsilon$ ): 255 (15,400), 285 (12,300), 345 (7,100). The infrared spectrum indicated diketone bands in the 6–7  $\mu$  region and a phthalide band at 5.75–5.80  $\mu$ . A single  $pK_a$  of 8.3 was found in 1:1 water-methanol. The material migrated as a single component ( $R_f$  0.80) in a 3% sodium arsenite paper chromatographic system<sup>19</sup> and also in a 1:1 water-methanol system ( $R_f$  0.60).

*Anal.* Calcd. for  $C_{22}H_{23}N_3ClO_3 \cdot H_2O$ : C, 53.17; H, 5.07; N, 5.64; Cl, 7.14;  $H_2O$ , 3.63. Found: C, 52.98; H, 5.00; N, 5.33; Cl, 7.41;  $H_2O$ , 4.19.

**Iso-7-chlorotetracycline.**—7-Chlorotetracycline hydrochloride was dissolved in 0.1 *N* aqueous sodium hydroxide and processed as was the 7-chloro-4-*epi*-tetracycline above, to yield iso-7-chlorotetracycline monohydrate in 68% yield,  $[\alpha]^{25}_D -97^\circ$  (0.5% in 0.03 *N* HCl), m.p. 213–224° dec. A single  $pK_a$  of 7.80 was found in 1:1 water-methanol.

*Anal.* Calcd. for  $C_{22}H_{23}N_3ClO_3 \cdot H_2O$ : C, 53.17; H, 5.07; N, 5.64; Cl, 7.14; water, 3.63. Found: C, 52.70; H, 5.20; N, 5.32; Cl, 7.14; water, 2.43.

Addition of concentrated hydrochloric acid to an acetone solution of the monohydrate yielded iso-7-chlorotetracycline hydrochloride,<sup>11</sup>  $[\alpha]^{25}_D -94.5^\circ$  (0.5% in 0.03 *N* HCl), m.p. 255–258° dec. A single  $pK_a$  of 7.80 was found in 1:1 water-methanol. The ultraviolet absorption spectrum in 0.1 *N* sodium hydroxide (aq.) (Fig. 2) showed  $\lambda_{max}$  in  $m\mu$  ( $\epsilon$ ): 255 (14,800), 285 (14,700), 345 (7,300). The ultraviolet and infrared spectra were identical with those of an authentic preparation.<sup>11</sup> The substance migrated as a single component ( $R_f$  0.80) in a 3% sodium arsenite paper chromatographic system<sup>19</sup> and also in a 1:1 water-methanol system ( $R_f$  0.60).

**Reversible Epimerization of Iso-7-chlorotetracycline and of Iso-7-chloro-4-*epi*-tetracycline.**—Repeated determinations of the ultraviolet absorption spectrum (in 0.1 *N* sodium hydroxide) of a 40  $\mu$ g./ml. solution of iso-7-chlorotetracycline in 2:1 dimethyl formamide-1 *M* aqueous  $NaH_2PO_4$  at 25° showed systematic changes occurring during the first 180 min. after preparation of the solution, with no additional changes occurring with further time. The ultraviolet absorption spectrum at equilibrium corresponded to a mixture of 32% iso-7-chlorotetracycline and 68% iso-7-chloro-4-*epi*-tetracycline.

The same sequence with iso-7-chloro-4-*epi*-tetracycline as the starting material yielded, after 120 min., a stable ultraviolet absorption spectrum corresponding to a mixture of 32% iso-7-chlorotetracycline and 68% iso-7-chloro-4-*epi*-tetracycline.

Figure 3 illustrates this approach to a common equilibrium mixture from both pure components.

**4-*epi*-Tetracycline from 7-Chloro-4-*epi*-tetracycline.**—A solution of 5 g. of 7-chloro-4-*epi*-tetracycline ammonium salt in 45 ml. of water containing 1.4 g. of 5% palladium-on-carbon was contacted with hydrogen on a Parr shaker. A rapid uptake of hydrogen ensued until, after 25 minutes, one equivalent of hydrogen was absorbed, at which time the rate decreased sharply. After filtration, the solution was diluted with 100 ml. of acetone, and the pH was adjusted to 9.0 with 1.0 ml. of concentrated ammonia water. Seeding induced the formation of crystalline ammonium salt which, after washing with acetone and drying *in vacuo* at 25°, weighed 2.60 g. The product had the ultraviolet absorption spectrum and microbiological assay of the ammonium salt of 4-*epi*-tetracycline.

**Benzenesulfonyl-4-*epi*-tetracyclonitrile Monohydrate.**—Twelve milliliters of benzenesulfonyl chloride was added dropwise with stirring to a mixture of 10 g. of 4-*epi*-tetracycline and 20 ml. of pyridine at 10° (ice-bath); the rate of addition was controlled so that the temperature did not exceed 40°. When the temperature began to fall, the reaction was continued for 30 min. without the ice-bath. One hundred ml. of ether was added with stirring, followed by settling and decantation of the supernatant. The thick, gummy residue was stirred with 500 ml. of water to yield an amorphous solid which was air-dried and slurried with 250 ml. of acetone to yield a yellow, crystalline, product which was filtered off, washed with acetone, and dried *in vacuo*. The product was dissolved in 15 ml. of dimethylformamide and 50 ml. of acetone was added. The resulting yellow, crystalline product, after drying *in vacuo*, weighed 5.2 g.,  $[\alpha]^{25}_D -336^\circ$  (0.5% in dimethylformamide), dec. at 200°. The ultraviolet absorption spectrum in 0.1 *N*  $H_2SO_4$  (aq.) containing 1% by volume of dimethylformamide showed  $\lambda_{max}$  in  $m\mu$  ( $\epsilon$ ): 270 (14,200), 347 (9,900) and  $\lambda_{min}$  in  $m\mu$  ( $\epsilon$ ): 310 (7,650). The infrared spectrum showed a nitrile band at 4.56  $\mu$ .

*Anal.* Calcd. for  $C_{25}H_{29}N_3SO_{10}$ : C, 57.40; H, 4.96; N, 4.78; S, 5.47. Found: C, 57.51; H, 5.29; N, 4.74; S, 4.97.

**Benzenesulfonyltetracyclonitrile Dimethylformamide Solvate.**—Twenty-four milliliters of benzenesulfonyl chloride was added dropwise with stirring to a mixture of 20 g. of tetracycline and 40 ml. of pyridine at 10° (ice-bath); the rate of addition was controlled so that the temperature did not exceed 50°. When the temperature began to fall, the reaction was continued for 30 min. without the ice-bath; 75 ml. of ether was added with stirring, followed by settling and decantation of the supernatant. To the thick, gummy residue was added 500 ml. of water; on stirring, the mixture was partly converted to a crystalline solid. The residue remaining after filtration was dried *in vacuo* and dissolved in 10 ml. of Ethyl Cellosolve containing 4.4 ml. of triethylamine (pH 9.5). After filtration, the solution was adjusted to pH 2.5 with concentrated HCl and allowed to remain on the rotary shaker overnight. The resulting crystalline product, after being washed with 10 ml. of Ethyl Cellosolve containing 1 ml. of 2 *N* HCl and being dried *in vacuo*, weighed 6.3 g. The product was recrystallized from 1:4 dimethylformamide-acetone,  $[\alpha]^{25}_D -416^\circ$  (0.5% in dimethylformamide), dec. at 210°. The ultraviolet absorption spectrum in 0.1 *N*  $H_2SO_4$  (aq.) containing 1% by volume of dimethylformamide showed  $\lambda_{max}$  in  $m\mu$  ( $\epsilon$ ): 275 (15,250), 348 (11,350) and  $\lambda_{min}$  in  $m\mu$  ( $\epsilon$ ): 319 (9,650). The infrared spectrum showed a nitrile band at 4.55  $\mu$ .

*Anal.* Calcd. for  $C_{31}H_{34}N_3SO_{10}$ : C, 58.20; H, 5.31; N, 6.56; S, 5.00. Found: C, 58.25; H, 5.24; N, 5.93; S, 4.93.

**7-Chloro-4-*epi*-tetracyclonitrile Monohydrate.**—Four milliliters of methanesulfonyl chloride was added dropwise with stirring to a mixture of 5.0 g. of 7-chloro-4-*epi*-tetracycline and 20 ml. of pyridine at –5° (ice-bath); the addition rate was controlled so that the temperature did not exceed 5°. The mixture was stirred 1 hr., and 150 ml. of ether was then added, followed by stirring, settling, and decantation of the supernatant. The solids were leached with 75 ml. of water at pH 1. The wet residue remaining after filtration was dissolved in 10 ml. of dimethylformamide, after which was added 30 ml. of chloroform. Crystalline nitrile precipitated which, after being washed with acetone, washed with ether, and dried *in vacuo*, weighed 1.5 g.,  $[\alpha]^{25}_D -300^\circ$  (0.5% in dimethylformamide), dec. at 200°. The ultraviolet absorption spectrum in 0.1 *N*  $H_2SO_4$  (aq.) containing 1% by volume of dimethylformamide showed  $\lambda_{max}$  in  $m\mu$  ( $\epsilon$ ): 272 (14,100), 366 (8,750) and  $\lambda_{min}$  in  $m\mu$  ( $\epsilon$ ): 320 (6,750). The infrared spectrum showed a nitrile band at 4.57  $\mu$ .

*Anal.* Calcd. for  $C_{22}H_{23}N_3O_3Cl$ : C, 55.10; H, 4.80; N, 5.85; Cl, 7.40. Found: C, 54.91; H, 4.76; N, 5.72; Cl, 7.89.

**7-Chlorotetracyclonitrile.**—Four milliliters of methanesulfonyl chloride was added dropwise with stirring to a mixture of 5.0 g. of 7-chlorotetracycline and 20 ml. of pyridine at –5° (ice-bath); the addition rate was controlled so that the temperature did not exceed 5°. The mixture was stirred 45 minutes and 100 ml. of water was then added with stirring. The yellow residue remaining after filtration was dissolved in dimethylformamide. Five volumes of acetone

(19) T. Berti and L. Cina, *Boll. Ist. Sieroterap. Milan.*, **33**, 643 (1954).



were added; the resulting crystalline product, after being washed with acetone and dried *in vacuo*, weighed 2.6 g.,  $[\alpha]_D^{25} -338^\circ$  (0.5% in dimethylformamide), dec. at  $220^\circ$ . The ultraviolet absorption spectrum in 0.1 *N*  $H_2SO_4$  (aq.) containing 1% by volume of dimethylformamide showed  $\lambda_{max}$  in  $m\mu$  ( $\epsilon$ ): 276 (14,100), 365 (9,350) and  $\lambda_{min}$  in  $m\mu$  ( $\epsilon$ ): 325 (7,250). The infrared spectrum showed a nitrile band at  $4.54 \mu$ .

*Anal.* Calcd. for  $C_{22}H_{21}N_2O_7Cl$ : C, 57.30; H, 4.56; N, 6.07; Cl, 7.69. Found: C, 56.99; H, 5.01; N, 6.37; Cl, 7.37.

**Attempted Epimerization of the Nitriles. A. Benzenesulfonyl-4-*epi*-tetracyclinonitrile.**—To a series of solutions (1 mg./ml.) of benzenesulfonyl-4-*epi*-tetracyclinonitrile in 4:1 dimethylformamide-water was added enough 85% orthophosphoric acid to give the following pH values: 0.3, 2.2, 2.6, 3.1, 3.5. In no case did aging for 114 hr. at  $25^\circ$  bring about any epimerization detectable by ultraviolet absorption spectrum changes. Both 4-*epi*-tetracycline and 7-chloro-4-*epi*-tetracycline were completely equilibrated in 24 hr. under the conditions of the pH 3.5 experiment above.

**B. 7-Chloro-4-*epi*-tetracyclinonitrile.**—A solution of 7-chloro-4-*epi*-tetracyclinonitrile (1 mg./ml.) in 4:1 dimethylformamide-water, brought to a pH of 1 with 85% orthophosphoric acid, was allowed to remain at  $25^\circ$  for 2 hr. and also at  $40^\circ$  for 44 hr. A solution of 7-chloro-4-*epi*-tetracyclinonitrile (1 mg./ml.) in 1:4 dimethylformamide-pH 7.0 phosphate buffer was allowed to remain at  $25^\circ$  for 22 hr. In no case could any epimerization be detected by ultraviolet absorption spectrum changes.

**C. 7-Chlorotetracyclinonitrile.**—To a series of solutions (1 mg./ml.) of 7-chlorotetracyclinonitrile in 4:1 dimethylformamide-water was added enough 85% orthophosphoric acid to give the following pH values: 0.3, 2.2, 2.6, 3.3. All solutions were aged at  $25^\circ$  for 22 hr. The pH 0.3 solution was heated to  $40^\circ$  for 22 hr. The pH 3.3 solution was heated to  $85^\circ$  for 3 hr. A solution of 7-chlorotetracyclinonitrile (1 mg./ml.) in 1:4 dimethylformamide-pH 7.0 phosphate buffer was allowed to remain at  $25^\circ$  for 22 hr. In no case could any epimerization be detected by ultraviolet absorption spectrum changes. Both 4-*epi*-tetracycline and 7-chloro-4-*epi*-tetracycline were completely equilibrated in 24 hr. under the conditions of the pH 3.5,  $25^\circ$  experiment above.

In the cases of all attempted nitrile equilibrations, the limits on the severity of the conditions tried were set by the appearance of other types of chemical changes.

**4-*epi*-Tetracycline Methiodide.**—A solution of 10 g. of 4-*epi*-tetracycline and 35 ml. of methyl iodide in 100 ml. of tetrahydrofuran was stirred for 24 hr. at room temperature. The resulting crystalline product was washed with 20 ml. of tetrahydrofuran and dried *in vacuo* at  $40^\circ$  to give a yield of 10.1 g. This was dissolved in 250 ml. of ethanol at  $50^\circ$ . Aging the solution with stirring at room temperature for 2 hr. gave a crop of crystalline methiodide contaminated with substantial amounts of unreacted 4-*epi*-tetracycline. The mother liquor was warmed to  $40^\circ$  and mixed with an equal volume of petroleum ether. On cooling, additional crystalline product precipitated which, after being washed with petroleum ether and dried, weighed 4.42 g.,  $[\alpha]_D^{25} -265^\circ$  (0.5% in 0.03 *N* HCl), m.p.  $161-162^\circ$  dec. The ultraviolet absorption spectrum in 0.1 *N*  $H_2SO_4$  (aq.) showed  $\lambda_{max}$  in  $m\mu$  ( $\epsilon$ ): 223 (21,900), 250 (15,100), 270 (15,000, shoulder), 355 (13,500).

*Anal.* Calcd. for  $C_{23}H_{27}N_2O_8I$ : C, 47.11; H, 4.64; N, 4.71; I, 21.64. Found: C, 47.32; H, 4.74; N, 4.64; I, 21.29.

**Tetracycline Methiodide.**—A solution of 30 g. of tetracycline and 105 ml. of methyl iodide in 450 ml. of tetrahydrofuran was stirred for 24 hr. at room temperature. The resulting crystalline product was washed with 50 ml. of tetrahydrofuran and dried *in vacuo* at  $40^\circ$  to give a yield of 34.5 g. Recrystallization from 750 ml. of ethanol ( $50^\circ$ ), washing with cold ethanol, and drying *in vacuo* yielded 19.3 g. of product,  $[\alpha]_D^{25} -198^\circ$  (0.5% in 0.03 *N* HCl), m.p.  $178-180^\circ$  dec. The ultraviolet absorption spectrum in 0.1 *N*  $H_2SO_4$  (aq.) showed  $\lambda_{max}$  in  $m\mu$  ( $\epsilon$ ): 223 (25,400), 274 (18,000), 355 (14,600).

*Anal.* Calcd. for  $C_{23}H_{27}N_2O_8I$ : C, 47.11; H, 4.64; N, 4.71; I, 21.64. Found: C, 47.38; H, 4.58; N, 4.91; I, 22.15.

**Reversible Epimerization of Tetracycline Methiodide and 4-*epi*-Tetracycline Methiodide.**—A solution of 49 mg. of 4-

*epi*-tetracycline methiodide in 1 ml. of 2:1 dimethylformamide-1 *M* aqueous  $NaH_2PO_4$  was allowed to remain 16.5 hr. at  $25^\circ$ . At the end of that time a crystalline solid had formed whose ultraviolet absorption spectrum showed it to be pure tetracycline methiodide. An AR assay of the mother liquor indicated a composition of 65% tetracycline methiodide and 35% 4-*epi*-tetracycline methiodide.

**Rapid Reversible Epimerization of Tetracycline and 4-*epi*-Tetracycline in Glacial Acetic Acid with Four Equivalents of Zinc Acetate (Simulated Reduction Solvent).**—A solution of 50 mg. of tetracycline and 50 mg. of zinc acetate dihydrate (4 equivalents) in 1 ml. of glacial acetic acid was allowed to remain at  $25^\circ$  while its ultraviolet absorption spectrum was determined repeatedly. After 20 min., the tetracycline was approximately 13% epimerized; after 140 min., approximately 35% epimerized. The same sequence starting with 4-*epi*-tetracycline showed 13% tetracycline after 20 min., 50% after 140 min.

**Slow Reversible Epimerization of Tetracycline Methiodide and 4-*epi*-Tetracycline Methiodide in 1:1 Acetic Acid-Water with Four Equivalents of Zinc Acetate (Simulated Reduction Solvent).**—A solution of 55 mg. of tetracycline methiodide and 38 mg. of zinc acetate dihydrate (4 equivalents) in 1.0 ml. of 1:1 acetic acid-water was allowed to remain at  $25^\circ$  while its ultraviolet absorption was repeatedly determined. After 260 min., essentially no change ( $\sim 3\%$  epimerization) had occurred. The same sequence starting with 4-*epi*-tetracycline methiodide yielded essentially no change after 120 min. and approximately 9% change in the direction of tetracycline methiodide after 260 min. Under these conditions the epimerization rates for the methiodides are measurably slower than those for the parent compounds (Table I).

**Desdimethylaminotetracycline. A. From Tetracycline Methiodide.**—A solution of 1.0 g. of tetracycline methiodide in 30 ml. of 1:1 acetic acid-water was purged with nitrogen; 1.5 g. of zinc dust was added and the mixture shaken under nitrogen for 30 min. and filtered. The filtrate was diluted with an equal volume of water and freeze-dried. The resulting solid was slurried 30 min. with 80 ml. of water containing 0.8 ml. of concentrated hydrochloric acid. The residue was washed with water, dried *in vacuo* (354 mg.), and dissolved in 3 ml. of methanol. Fifty milliliters of ether was added and the resulting precipitate removed by filtration. The filtrate was evaporated to about 1 ml. and triturated with 3 ml. of water. Crystals formed which after water washing and drying weighed 270 mg. Recrystallization from warm methanol yielded desdimethylaminotetracycline,  $[\alpha]_D^{25} -250^\circ$  (0.5% in Methyl Cellosolve), m.p.  $195^\circ$  dec. The ultraviolet absorption spectrum in 0.1 *N* methanolic sulfuric acid showed  $\lambda_{max}$  in  $m\mu$  ( $\epsilon$ ): 221 (23,100), 263 (24,700), 360 (21,400).

*Anal.* Calcd. for  $C_{20}H_{19}NO_8$ : C, 59.75; H, 4.77; N, 3.49. Found: C, 59.80; H, 4.72; N, 3.30.

**B. From 4-*epi*-Tetracycline Methiodide.**—Three grams of 4-*epi*-tetracycline methiodide was reduced, freeze-dried, leached with hydrochloric acid and dried *in vacuo*, using the conditions described above for tetracycline methiodide, to yield 2.4 g. of product. Two grams of this material was extracted with 100 ml. of hot ethyl acetate, leaving 0.76 g. of unextractables consisting largely of unreacted methiodide. Dilution of the extract with four volumes of petroleum ether yielded a precipitate which on drying weighed 0.9 g. A solution of this precipitate in 5 ml. of warm methanol gave, after one hour aging at room temperature, a crystalline product which, after being washed with methanol and dried, weighed 0.23 g.,  $[\alpha]_D^{25} -251^\circ$  (0.5% in Methyl Cellosolve), m.p.  $195^\circ$  dec. Mixed melting point with the product from tetracycline methiodide showed no depression. The ultraviolet absorption spectrum in 0.1 *N* methanolic sulfuric acid showed  $\lambda_{max}$  in  $m\mu$  ( $\epsilon$ ): 221 (22,200), 263 (24,300), 360 (21,300).

*Anal.* Calcd. for  $C_{20}H_{19}NO_8$ : C, 59.75; H, 4.77; N, 3.49. Found: C, 59.40; H, 4.88; N, 3.42.

**Identity and Homogeneity of Desdimethylaminotetracycline from Tetracycline Methiodide and from 4-*epi*-Tetracycline Methiodide.**—The samples of desdimethylaminotetracycline prepared from the two sources were judged identical on the basis of optical rotation, melting point, ultraviolet absorption spectrum and the elemental analysis data presented above. In addition, infrared spectra were identical.

Countercurrent distribution in a 50-tube Craig machine, using the system chloroform against 0.2 *M* aqueous phosphate buffer (*pH* 7.53), gave, for each sample, a single peak at tube 34. The shape of the peak for each sample corresponded with the theoretical curve for a single component

peaking at tube 34. The ultraviolet absorption spectra of both peak tube contents were identical with each other and with those of the two starting materials.

PEARL RIVER, NEW YORK

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY OF THE UNIVERSITY OF CALIFORNIA AT LOS ANGELES]

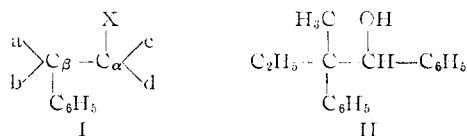
## Studies in Stereochemistry. XXVI. Solvolytic Rearrangements in the 1,2-Diphenyl-2-methyl-1-butanol System<sup>1</sup>

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The tosylates of the optically pure diastereomers of the 1,2-diphenyl-2-methyl-1-butanol system have been solvolyzed in dry acetic and formic acids, respectively. Phenyl migration from *C*<sub>β</sub> dominates the reaction course, at the most only a trace of alkyl migration being observed. In the more nucleophilic and poorer ionizing solvent (acetic acid), a larger amount of unrearranged product was observed, the simple substitution reaction being somewhat stereospecific in the *direction of inversion*. In the less nucleophilic and better ionizing solvent (formic acid), a smaller amount of unrearranged product was observed, the simple substitution reaction being somewhat stereospecific in the *direction of retention*. The results indicate that three stereochemically discrete processes compete with one another, all three leading to simple substitution product: (1) a simple solvolytic inversion mechanism; (2) a simple solvolytic mechanism whose specificity is sterically induced by the asymmetry at *C*<sub>β</sub>; (3) a phenonium ion mechanism which can lead to simple substitution product with retention of configuration. That rearranged products arise from both bridged and rearranged open carbonium ions is indicated.

In previous investigations of this series, the solvolytic behavior of systems I have been studied in which *a* = *c* = alkyl and *b* = *d* = hydrogen<sup>2</sup>; *a* = *b* = *c* = *d* = alkyl<sup>3</sup>; *a* = alkyl, *b* = *c* = hydrogen and *d* = phenyl<sup>4</sup>; and *a* = phenyl, *b* = *c* = hydrogen and *d* = alkyl.<sup>5</sup> Others have studied systems I in which *a* = alkyl, *b* = *c* = *d* = hydrogen<sup>6</sup>; *a* = *b* = *c* = hydrogen and *d* = alkyl<sup>7</sup>; *a* = *c* = phenyl, *b* = *d* = hydrogen.<sup>8</sup> The tendency for phenyl to participate in ionization was found to vary markedly with the electrical nature of substituents at *C*<sub>α</sub> and *C*<sub>β</sub>, as well as the character of the solvent, and the steric situation within the molecule. The 1,2-diphenyl-2-methyl-1-butanol



system (II) belongs in a borderline category with respect to phenyl involvement in ionization, and this paper reports the results of an investigation of the acetolysis and formolysis of its diastereomeric tosylates.

### Methods

The four stereoisomers of II were isolated in a pure state and their configurations assigned in a previous investigation.<sup>9</sup> The four tosylate deriva-

(1) This work was sponsored by the Office of Ordnance Research, U. S. Army.

(2) (a) D. J. Cram, *THIS JOURNAL*, **71**, 3863 (1949); (b) **71**, 3875 (1949); (c) **74**, 2129 (1952); (d) **74**, 2159 (1952); (e) D. J. Cram and F. A. Abd Elhafez, *ibid.*, **75**, 3189 (1953); (f) D. J. Cram, *ibid.*, **75**, 332 (1953).

(3) D. J. Cram and J. D. Knight, *ibid.*, **74**, 5839 (1952).

(4) D. J. Cram and F. A. Abd Elhafez, *ibid.*, **75**, 339 (1953).

(5) D. J. Cram and F. A. Abd Elhafez, *ibid.*, **76**, 28 (1954).

(6) S. Winstein and K. C. Schreiber, *ibid.*, **74**, 2171 (1952).

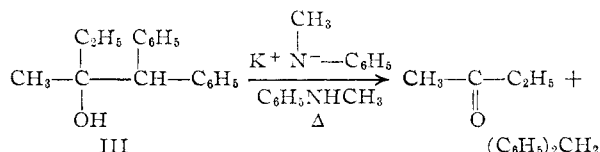
(7) S. Winstein, M. Brown, K. C. Schreiber and A. H. Schlesinger, *ibid.*, **74**, 1140 (1952).

(8) C. J. Collins and W. A. Bonner, *ibid.*, **77**, 92 (1955).

(9) D. J. Cram and J. Allinger, *ibid.*, **76**, 4516 (1954). The meaning of the configurational terms *threo* and *erythro* as applied to system II are defined in this reference.

tives were prepared from the corresponding potassium alcoholates and tosyl chloride, the derivatives possessing the *threo* configuration being particularly unstable. The products were isolated in the usual way, the olefin-ester mixtures being converted ( $\text{LiAlH}_4$ ) to alcohol-olefin mixtures which were separated into olefin and alcohol components. In runs involving *erythro* materials and *threo* materials, 73 and 87%, respectively, of the starting materials were accountable.

**Analysis of Alcohol Fractions.**—Comparison of the infrared spectra of the alcohol components obtained from the solvolyses with those of *threo*- and *erythro*-II and of III (product of phenyl migration) suggested the bulk of the material to consist of these components. Accordingly, infrared analytical procedures were developed based on these three components utilizing the six most advantageous wave lengths in the infrared for analysis. Two independent sets of three simultaneous equations when solved provided estimates of percentage composition. Known mixtures of the three components indicated the deviations from Beer's law to be small. The analyses for the acetolysis runs (runs 1 and 2 of Table I) for the total secondary alcohol (diastereomers of II) and ter-



tiary alcohol III have a probable error of about 2% since the optical densities differ by factors from 1 to 4. The analyses for the individual diastereomers of II are grossly inaccurate because the optical densities of these components are rather close together, the biggest factor being 1.6. To improve the accuracy of this analysis and to examine the optical character of the diastereomeric components (II), the alcohol mixtures were heated with strong