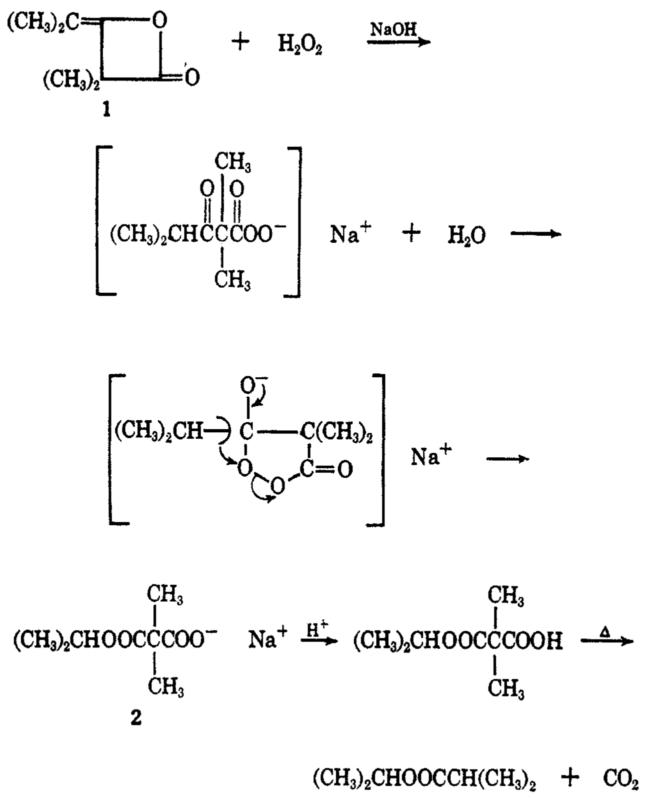
Scheme I



and 4 were considered less plausible than those proposed because the nucleophilic attack on 1 has been found to occur consistently at the carbonyl carbon atom[1,2] in contrast to that on saturated β-lactones. Furthermore, 3 is also an intermediate in the formation of the epoxide, which was not found and which would not be expected to rearrange to 2. It is also reasonable to expect that the rearrangement of 4 would involve the same intermediates as for the proposed mechanism.

Experimental Section

Reaction of Hydrogen Peroxide with 3-Hydroxy-2,2,4-trimethyl-3-pentenoic Acid β-Lactone (1).—Into a 4-l. beaker outfitted with a stirrer, a thermometer, and electrodes for a pH meter were placed 600 ml of water and 180 g of a 30% solution of hydrogen peroxide. The mixture was stirred and maintained at pH 8 to 9 during the gradual concurrent addition of 200 g of 3-hydroxy-2,2,4-trimethyl-3-pentenoic acid β-lactone[1] and 1400 ml of 1 *N* sodium hydroxide. The temperature was kept at 30 to 40° by cooling the mixture with ice during the addition period of 1 hr. After the addition step the mixture was stirred for 15 min. A sample of the mixture was titrated with potassium permanganate and then with potassium iodide. A trace of hydrogen peroxide was found, but the presence of a peroxy acid was not indicated.

The mixture was acidified to pH 1.0 with 20% hydrochloric acid, and the product was extracted with four 200-ml portions of pentane. The extract was washed with three 50-ml portions of water and then evaporated *in vacuo* at room temperature to a small volume in a rotary flash evaporator. The yield of isopropyl hydrogen dimethylmalonate was 176 g (72%). *Anal.* Calcd for $C_8H_{14}O_4$: neut equiv, 174. Found: neut equiv, 186. Infrared absorption was at 5.9 μ (broad).

An attempt was made to distil the product *in vacuo* but decomposition began at about 50°. Therefore, the product was destructively distilled at atmospheric pressure, and a gas, noncondensable in Dry Ice, was evolved. The gas was passed into a solution of calcium hydroxide. A precipitate formed which redissolved upon continued bubbling of the gas through the mixture. The distillate was redistilled to yield 108 g of isopropyl isobutyrate (82% from 2), bp 118–120°.[3] The infrared spectrum of this product was identical with that of an authentic sample of isopropyl isobutyrate.

Hydrolytic Stability of 1.—A mixture of 20.0 g (0.14 mole) of 1 and 100 ml of water was stirred vigorously and maintained at 30° for 30 min. During this time the pH of the mixture was maintained at 8.0 by gradual addition of 0.1 *N* sodium hydroxide. The amount of base consumed was 3.5 ml (0.35 mequiv), corresponding to 0.25% hydrolysis.

Registry No.—**1**, 3173-79-3; isopropyl hydrogen dimethylmalonate, 7695-26-3.

[3] S. Young and E. C. Fortey, *J. Chem. Soc.*, **81**, 785 (1902).

Chemistry of Tetracyclines. I. Mercuric Acetate Oxidation of Tetracycline

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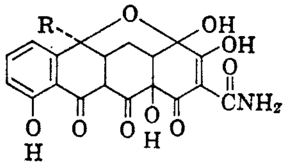
Blackwood and Stephens[1] have reported the oxidation of tetracycline hydrochloride with N-chlorosuccinimide in water to the hemiketal (I), and Esse and co-workers[2] have reported the oxidation of 6-demethyltetracycline with chloric acid in acetic acid to the analogous hemiketal (II).



I, $R = CH_3$
II, $R = H$

In an attempt to accomplish the same transformation, we have treated tetracycline base (IV) in acetic acid with mercuric acetate. Crystallization of the product produced instead the dimethylamine salt of quinone VII as the only isolable product, the free quinone VII being isolated on acidic work-up of the reaction. The structure of VII was indicated by infrared bands in the hydroxyl (2.95 μ) and carbonyl (γ-lactone, 5.62 μ) regions and by ultraviolet absorption maxima (Table I) characteristic for quinone [299 mμ (ε 18,900), 422 mμ (ε 490)] and 8-hydroxy-1-tetralone [340 mμ (ε 5500)] chromophores. When VII was reductively acetylated, pentaacetate VIII was produced. As expected, for this structure, the 10-acetoxyl and the two pairs of symmetrically oriented acetoxyls on ring A gave rise to a three-proton singlet and two six-proton singlets in the appropriate region of the nmr spectrum.

The sequence of events leading from tetracycline (IV) to quinone VII is believed to be that depicted in Scheme I. Since tetracycline is stable at room temperature in glacial acetic acid with or without added

[1] R. K. Blackwood and C. R. Stephens, *Can. J. Chem.*, **43**, 1382 (1965); *J. Am. Chem. Soc.*, **86**, 2736 (1964).

[2] R. C. Esse, J. A. Lowery, C. R. Tamorria, and G. M. Sieger, *ibid.*, **86**, 3874 (1964).

SCHEME I

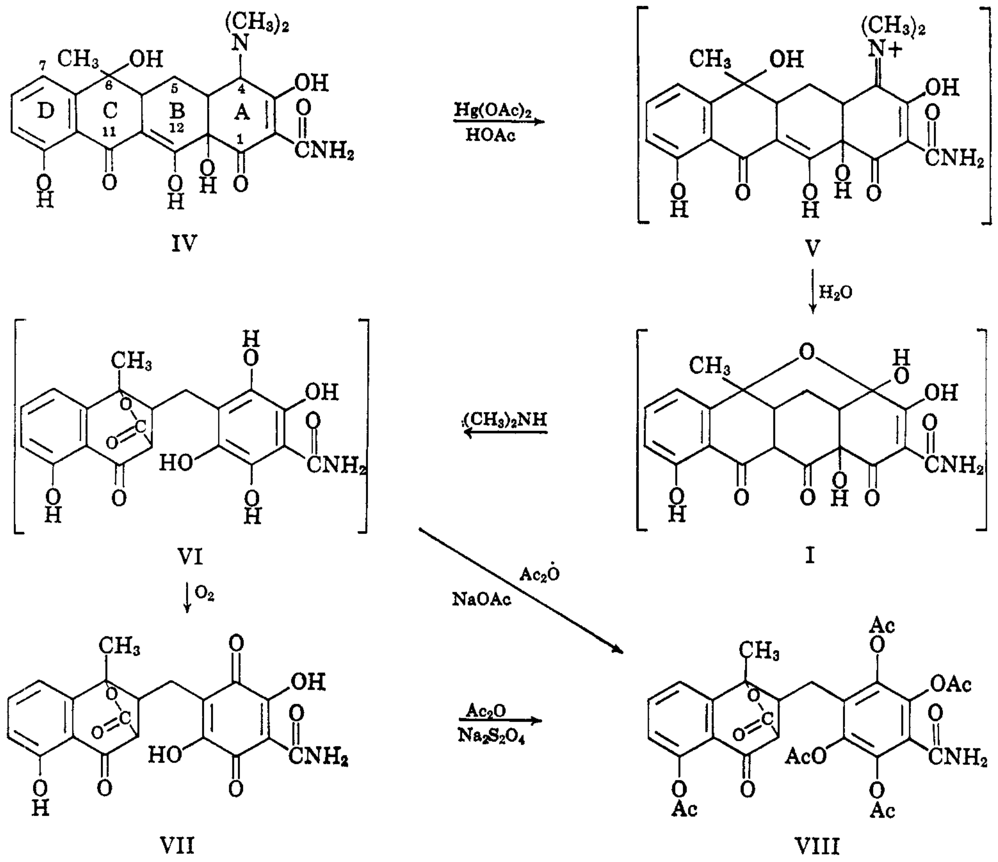


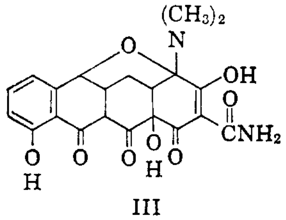
TABLE I

ULTRAVIOLET SPECTRA

Compd (no.)	Solvent	λ_{max}, mμ (ε)
Yellow quinone (VII)	MeOH-0.01 *N* HCl	274 (29,600), 296 (18,300), 340 (5500) visible not taken
	MeOH-0.01 *N* KOH	271 (17,900), 308 (15,500), sh 314 (14,700), 389 (8600)
8-Hydroxy-1-tetralone	Acidic ethanol	260 (9300), 335 (3100)[a]
2,5-Dihydroxybenzoquinone	Ethanol-0.01 *N* HCl	284 (22,600), 382 (320)
	Ethanol-0.01 *N* KOH	314 (26,800), 323 (26,900), visible not taken

[a] F. A. Hochstein, C. R. Stevens, L. H. Conover, P. P. Regna, R. P. Pasternack, P. N. Gordon, F. J. Pilgrim, K. J. Brunings, and R. B. Woodward, *J. Am. Chem. Soc.*, **75**, 5455 (1953).

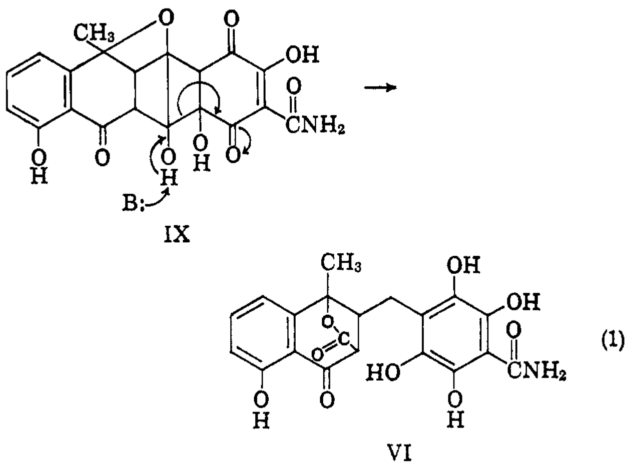
triethylamine or sodium acetate, the first step must be mercuric acetate oxidation of the dimethylamino group to produce imine V. Esse and co-workers[2] have proposed an analogous imine as an intermediate in the reaction of 6-demethyltetracycline with mercuric acetate in DMF to the bridged compound (III).

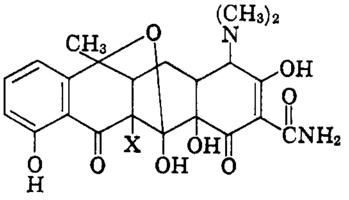


III

The imine is rapidly hydrolyzed by the water present in the glacial acetic acid (Karl Fisher analysis 0.39% water) to the ketone which exists predominately as the hemiketal (I).

A sample of I was prepared by the method of Blackwood and Stephens.[1] This compound was recovered unchanged from glacial acetic acid with or without added mercuric acetate but was found to produce the quinone VII when dimethylamine was added to the acetic acid solution. This establishes the ring opening as a base-catalyzed reaction. The mechanism of this ring opening (eq 1) is suggested to involve IX, a tautomer of hemiketal I. The existence of IX, at least in





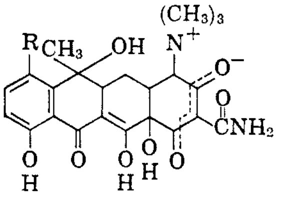
Xa, X = Cl
b, X = F

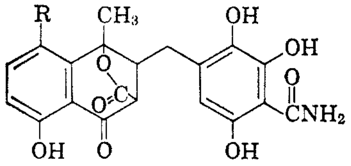
small amounts, is not unlikely in view of the fact that 11a-halo derivatives Xa and Xb are stable in the bridged forms depicted.[3] In addition, the intermediate hydroquinone VI was isolated as its pentaacetate VIII when acetic anhydride was added to the reaction mixture at an early stage.

The last step is clearly air oxidation of VI to the quinone VII which, of course, is isolated as a salt with the dimethylamine generated earlier in the sequence.

The fact that the published procedures for producing hemiketals I and II involve acidic conditions explains why lactonic quinones, such as described above, have not been found among the products. Lack of a base catalyst would prevent the B-ring cleavage required to produce these new compounds.

The only reference in the literature to tetracycline derivatives isolated possessing lactones between 6 and 12 appeared when Hlavka, Bitha and Boothe[4] reported preparing lactones XIIa and XIIb by heating betaines XIa and XIb, but they did not prepare the quinones from these products.


XIa, R = H
b, R = Cl


XIIa, R = H
b, R = Cl

Experimental Section

All melting points are uncorrected and were taken in evacuated capillary tubes. All infrared spectra were taken on a Perkin-Elmer Infracord, and the significant bands between 2.4 and 6.5 μ are recorded. All ultraviolet spectra were taken on a Perkin-Elmer 202 spectrophotometer. The microanalyses were performed by the Microanalytical Laboratory of the Squibb Instiute for Medical Research.

Quinone VII. A. Free Quinone.—Tetracycline (10.0 g, 22.5 mmoles) in 75 ml of glacial acetic acid and mercuric acetate (18.1 g, 56.7 mmoles) in 350 ml of the same solvent were mixed, and the resulting solution was allowed to stand at room temperature for 1 hr. After removal of the precipitated mercurous acetate by filtration, the reaction mixture was treated with excess hydrogen sulfide and the mercuric sulfide was removed by filtration through Celite. The filtrate was freeze dried, and the residue, after addition of 250 ml of water, was acidified with dilute HCl to pH 1.3. After stirring the mixture for 1 hr, the red-brown, amorphous solid was collected on a filter. This crude product was dissolved in 150 ml of acetone, and 450 ml. of methanol was added. The solution was concentrated until crystallization occurred. The dark yellow needles were collected on a filter, washed well with methanol, and dried (3.17 g 35%): mp (evacuated capillary) 262–264° dec. Recrystallization from acetone–methanol raised the melting point to 265–266° dec. However, recrystallization from methanol produced a polymorphic modification whose melting point (evacuated capillary) was 225.5–227.5° dec in the analytical sample: $[\alpha]^{20}\mathrm{D}$ −74 ± 11° (*c* 0.2, methanol); ultraviolet (methanol) 275 mμ (ε 22,400), 299 mμ (ε 18,400); ultraviolet (0.01 *N* methanolic hydrochloric acid) 274 mμ (ε 29,600), 296 (18,300), 340 (5500), visible peak not recorded; ultraviolet (0.01 *N* ethanolic sodium hydroxide) 271 mμ (ε 17,900), 308 (15,500) sh, 314 (14,700), 389 (8600), visible peak not recorded; infrared (mineral oil mull) 2.95 (m), 3.06 (m), 5.62 (s), 6.08 (s), 6.17 (w), 6.30 μ (m).

Anal. Calcd for $C_{20}H_{15}NO_9 \cdot H_2O$: C, 55.69; H, 3.97; N, 3.25. Found: C, 55.76; H, 3.95; N, 3.25.

B. Dimethylamine Salt of VII.—Beginning with 3.85 g of tetracycline, procedure 1 outlined above was followed to the completion of the freeze drying. The residue was triturated with 250 ml of cold methanol. The red, crystalline residue of dimethylamine salt weighed 1.05 g. The triturate was refrigerated to produce another crop of crystals (0.37 g). The infrared spectra of these two crops showed them to be virtually pure product (total yield 1.42 g, 35.8%). The analytical sample was prepared by crystallization several times from methanol: decomposes without melting between 200 and 250°; ultraviolet (methanol) 373 mμ (ε 19,200), 304 (21,300), 514 (690); infrared (mineral oil mull) 2.93 (s), 3.03 (w), 3.62 (m), 4.06 (m), 5.61 (s), 6.08 (s), 6.22 μ (w).

Anal. Calcd for $C_{22}H_{22}N_2O_9$: C, 57.64; H, 4.84; N, 6.11; neut equiv, 458. Found: C, 57.77; H, 4.49; N, 5.96; neut equiv, 458.

Alternatively, quinone VII (50 mg, 0.12 mmole) dissolved in 30 ml of methanol was allowed to react with 20 drops of dimethylamine. The solution turned light red but no precipitate formed. After concentration to 0.5 ml the solution remained free of any precipitate. Upon addition of 4 drops of acetic acid together with a few seeds, the solution deposited dark red crystals (33 mg 59%). The infrared spectrum was identical with that of the sample prepared under the first procedure described.

Pentaacetate VIII. A.—Tetracycline (1.00 g, 2.25 mmoles) in 3 ml of glacial acetic acid and mercuric acetate (2.00 g, 6.27 mmoles) in 32 ml of the same solvent were mixed and the solution allowed to stand under nitrogen for 1 hr. During this time a copious precipitate of mercurous acetate formed. Potassium acetate (fused, 0.7 g, 7 mmoles) and 50 ml of acetic anhydride were added, and the resulting mixture was stirred magnetically for 7 days under nitrogen. The mixture was treated with excess hydrogen sulfide, and the precipitate of mercuric sulfide was removed by filtration through Celite. The filtrate was evaporated under vacuum at 50°. The resulting oil was treated with water and, after 3 hr, the green powder formed was collected on a filter. The dried powder (1.27 g) was crystallized from methanol several times. Second and third crops were taken and recrystallized as needed. The pure samples were combined to give a total yield of 410 mg (34%), softens at 197°, melts at 200–202° (evaculated capillary). The infrared spectrum of this product was identical with that of the analytical sample prepared by further crystallization from methanol: mp (evacuated capillary) 201.5–202.5°; $[\alpha]^{20}\mathrm{D}$ −24.5° (*c* 0.47, methanol); ultraviolet (95% ethanol) 271 mμ (ε 8000), 343 mμ (ε, 4500); infrared (mineral oil mull) 3.05–3.13 (w), 5.58 (s) 5.80 (m), 5.86 (s), 6.05 (m), 6.15 (m), 6.30 μ (w).

Anal. Calcd for $C_{30}H_{27}NO_{14}$: C, 57.60; H, 4.35; OAc, 34.40. Found: C, 57.90; H, 4.34; OAc, 34.44.

B.—To a well-stirred solution of quinone VII (500 mg, 1.21 mmoles) in 60 ml of acetic anhydride under nitrogen, sodium hydrosulfite was added in four 1-g portions. Each addition was followed by the addition of 1.5 ml of 1 *N* hydrochloric acid, the color of the reaction mixture turning quickly to cherry red. To complete the solution of the hydrosulfite, an additional 3 ml of 1 *N* hydrochloric acid was required. The color during this time gradually faded to a pale tan. Fused sodium acetate was then added and within a short time enough heat was generated to cause the solution to reflux. The reflux rate was controlled by cooling the reaction flask in an ice bath periodically. After the solution, now colorless, had cooled, the flask was flushed with nitrogen, stoppered, and stirred for 3 days. The mixture was taken to dryness under vacuum at 50° and the residue was shaken with water for 3 hr. The white, crystalline product was collected on a filter [788 mg, mp (evacuated capillary) 184–200°] and subsequently recrystallized twice from methanol [603 mg, 43%, mp (evacuated capillary) 198–200°]. This product was ultimately shown to be identical with the pentaacetate prepared directly from tetracycline (in method A) by comparison of infrared spectra and by absence of a mixture melting point depression.

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(3) R. K. Blackwood, J. J. Beereboom, H. H. Rennhard, M. S. von Wittenau, and C. R. Stephens, *J. Am. Chem. Soc.*, **85**, 3943 (1963).

(4) J. J. Hlavka, P. Bitha, and J. H. Boothe, *ibid.*, **87**, 1795 (1965).

Stability of Hemiketal I in Acetic Acid under Various Conditions. A.—4-Oxo-4-dedimethylaminotetracycline 4,6-hemiketal (I, 207 mg, 0.499 mmole) was dissolved in 13 ml of glacial acetic acid, and the resulting solution was allowed to stand at room temperature for 1 hr. At the end of this time the solution was treated with hydrogen sulfide for a few minutes and then freeze dried overnight. The resulting amorphous, yellow powder was suspended in water for 2 hr. The mixture was adjusted to pH 2 with dilute hydrochloric acid and filtered to give an amorphous, yellow powder (159 mg, 77% recovery), $[\alpha]^{34}\text{D}$ −111° (*c* 0.4, methanol). The ultraviolet and infrared spectra were identical with those of the starting material.

B.—The procedure in A was repeated except that mercuric acetate (160 mg, 0.5 mole/mole of hemiketal) was incorporated at the beginning also. The product in this case was on amorphous, yellow powder (142 mg, 69% recovery), $[\alpha]^{34}\text{D}$ −113° (*c* 0.4, methanol). The ultraviolet and infrared spectra were identical with those of the starting material.

C.—The procedure in A was repeated except that mercuric acetate (160 mg, 0.5 mole/mole of hemiketal) and dimethylamine hydrochloride[5] (40 mg, 0.5 mole/mole of hemiketal) were also incorporated at the beginning. The product in this case was an amorphous, brown powder (147 mg). The infrared spectrum in this case was quite different from that of the starting hemiketal, having a strong γ-lactone band at 5.64 μ (mineral oil mull). Three crystallizations from methanol gave yellow crystal (27 mg, 13%). The ultraviolet and infrared spectra were identical with those of pure quinone VII.

D.—The procedure in A was repeated except that triethylamine (42 mg, 0.5 mole/mole of hemiketal) was also incorporated at the beginning. The product was an amorphous, orange-brown powder (147 mg). The infrared spectrum in this case was also quite different from that of the starting material, having a lactone band at 5.64 μ (mineral oil mull). Three crystallizations from methanol gave yellow crystals (29 mg, 14%), mp (evacuated capillary) 227.0–227.5° dec. The ultraviolet and infrared spectra were identical with those of pure quinone VII.

Stability of Tetracycline to Acetic Acid, Potassium Acetate, and Triethylamine.—Tetracycline (444 mg, 1 mmole), fused potassium acetate (98 mg, 1 mmole), and triethylamine (101 mg, 1 mmole) were dissolved in 7 ml of glacial acetic acid. After standing for 90 min at room temperature, the solution was sampled for paper chromatography (Whatman No. 1 paper buffered to pH 4.5 with McIlvaine's buffer, prewetted with 4:1 acetone–water, and developed with wet ethyl acetate). The only spot seen upon development was that of tetracycline (ultraviolet absorbing becoming ultraviolet green fluorescing on exposure to ammonia vapor).

Registry No.—Mercuric acetate, 1600-27-7; tetracycline, 60-54-8; VII, 7700-13-2; 8-hydroxy-1-tetralone, 7700-14-3; 2,5-dihydroxybenzoquinone, 615-94-1; dimethylamine salt of VII, 7700-15-4; VIII, 7703-94-8; I, 3764-23-6.

[5] Dimethylamine base is released in solution because mercuric ion complexes with chloride ion in preference to acetate ion thus effectively removing the hydrochloric acid.

Chemistry of Tetracyclines. II. Allotetracycline and Allooxytetracycline

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Compounds in the tetracycline family when treated with aqueous base under mild conditions are isomerized to phthalid derivatives called isotetracyclines.[1] This change is exemplified here by the isomerization of tetracycline (Ia) to isotetracycline (II) and is initiated by nucleophilic attack by base on C-11. We have found that, by changing the nature of the solvent and base, isomerization takes a different course.

When tetracycline and triethylamine were refluxed in dioxane, the tetracycline was slowly converted to an isomer which was named allotetracycline. The infrared band at 5.66 μ showed that this new compound possessed a γ-lactone function. Since the ultraviolet spectra in acid and in base (Table I) indicated the retention of the ring A chromophore and the presence of an 8-hydroxy-1-tetralone chromophore (especially compare with the ultraviolet spectra of V), the lactone must have been formed from the C-6 hydroxyl together with the carbonyl group at C-12, the only carbonyl group not involved in the chromophores. Allotetracycline is, therefore, IVa. Additional support for this structure is obtained by the fact that quinone V[2] is obtained from allotetracycline by mercuric acetate oxidation.

The ring cleavage and lactone formation that tetracycline undergoes to form allotetracycline is envisioned to proceed by the mechanism of base attack on tetracycline tautomer III analogous to that proposed for conversion of VI to VII.[2] The fact that more vigorous conditions are required to isomerize tetracycline to allotetracycline than to convert VI to VII is ascribed to the lack of developing aromaticity to promote the reaction in the former case.

When this reaction was extended to oxytetracycline (Ib), only a small yield of the expected allooxytetracycline was produced, the major portion of the product mixture being composed of nonbasic compounds. No explanation is offered at this time to explain the difference in reactivity between tetracycline and oxytetracycline in this reaction (see Scheme I).

Experimental Section

All melting points were taken in capillary tubes and are uncorrected. All infrared spectra were taken on a Perkin-Elmer Infracord and the significant bands between 2.4 and 6.5 μ are recorded. All ultraviolet spectra were taken on a Perkin-Elmer 202 spectrophotometer. The microanalyses were performed by the Microanalytical Laboratory of the Squibb Institute for Medical Research.

Allotetracycline (IVa).—Tetracycline (11.7 g, 26.3 mmoles) and triethylamine (4.0 ml, 30 mmoles) were dissolved in 60 ml of dioxane, and the resulting solution was refluxed under nitrogen for 18 hr. At this time paper chromatography[3] of an aliquot of the reaction mixture disclosed that the tetracycline had been completely isomerized.[4] The solvent was removed by distillation under vacuum, and the dark residue was dissolved in a minimum volume of boiling acetone. Fifty milliliters of benzene was added, and the solution was concentrated to about 25 ml to

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[1] (a) C. W. Waller, B. L. Hutching, C. F. Wolf, A. A. Goldman, R. W. Broschard, and J. H. Williams, *J. Am. Chem. Soc.*, **74**, 4981 (1952); (b) J. H. Boothe, J. Morton, II, J. P. Petisi, R. G. Wilkinson, and J. H. Williams, *Antibiot. Ann.*, 46 (1953); (c) P. Sensi, G. A. DeFerrair, G. G. Gallo, and G. Rolland, *Farmaco* (Pavia) *Ed. Sci.*, **10**, 337 (1955); (d) J. R. D. McCormack, S. M. Fox, L. L. Smith, B. A. Bitler, J. Reichenthal, V. E. Arigoni, W. H. Muller, R. Winterbottom, and A. P. Doerschuk, *J. Am. Chem. Soc.*, **79**, 2849 (1957); (e) C. R. Stephens, L. H. Conover, R. Pasternack, F. A. Hochstein, W. T. Moreland, P. P. Regna, F. J. Pilgrim, K. J. Brunnings, and R. B. Woodward, *ibid.*, **76**, 3568 (1954); (f) F. A. Hochstein, C. R. Stephens, L. H. Conover, P. P. Regna, R. Pasternack, P. N. Gordon, F. J. Pilgrim, K. J. Brunnings, and R. B. Woodward, *ibid.*, **75**, 5455 (1953).

[2] See footnote *c* of Table I.

[3] Whatman No. 1 paper was treated with McIlvaine's buffer and dried. The paper was remoistened with 4:1 acetone–water, the acetone being allowed to evaporate prior to developing the chromatogram with wet ethyl acetate.

[4] Tetracycline is detected at R_f ~0.4 as an absorbing spot under ultraviolet light. Allotetracycline is detected at R_f ~0.95 as a blue-white fluorescing spot under ultraviolet light.