SOME TRANSFORMATIONS OF TETRACYCLINE AT THE 4-POSITION[1]

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Dedicated to Professor R. B. Sandin on the Occasion of his Sixty-Eighth Birthday

ABSTRACT

Reactions leading to the replacement of the 4-dimethylamino group of tetracycline by keto, hydroxy, hydroxyimino, hydrozono, and amino groups are reported.

INTRODUCTION

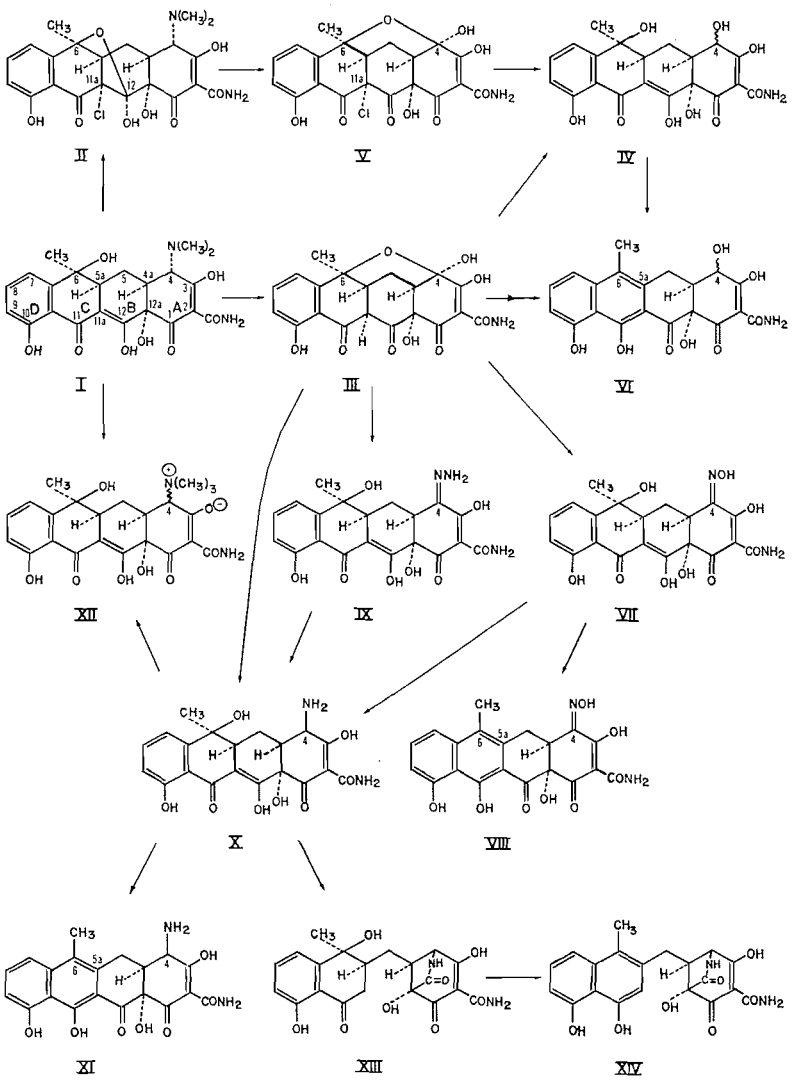
Only a limited number of transformations of tetracycline at C-4 have been previously reported: 4-dedimethylaminotetracyclines (3, 4), 4-epitetracyclines (5), and tetracycline methiodides (4, 6). An extension of earlier halogenation studies (7) has provided an entrée to further modification at this position. The chemistry investigated should be adaptable to the preparation of a broad variety of related derivatives.

DISCUSSION

The reaction of *amphoteric* tetracycline (I) with N-chlorosuccinimide in aqueous solvents to yield 11*a*-chlorotetracycline-6,12-hemiketal (II) has been described (7). A less-than-drastic change in reaction conditions—treatment of tetracycline *hydrochloride* with N-chlorosuccinimide in water—leads to rapid precipitation of a very different product, which we have characterized as 4-oxo-4-dedimethylaminotetracycline-4,6-hemiketal (III). Carbonyl absorption at 5.7 μ and ultraviolet absorption in acid (8-hydroxytetralone and unmodified A-ring chromophores) at first suggested possible close structural analogy to 11*a*-substituted tetracyclines (e.g. XV, cf. ref. 7). However, in excess methanolic sodium hydroxide, the ultraviolet absorption indicated that III is enolic, suggesting alternative analogy to 12*a*-epi-4-dedimethylamino-5-hydroxytetracycline (XVI, cf. refs. 6 and 8), which is isolated in the completely ketonic form, but is also enolized under basic conditions. Analyses on III confirmed suspected loss of the dimethylamine, and, further, showed that it contains additional oxygen, *but no chlorine*. The chemical properties of III, detailed below, demonstrate the presence, or potential presence, of a ketone group at C-4 and retention of the *cis* A- to B-ring juncture. No additional infrared absorption beyond that seen with compounds such as XV and XVI is noted in the carbonyl region. This and the unmodified A-ring ultraviolet chromophore in acid lead to the conclusion that the C-4 ketone is not free. By employment of the now firmly established stereochemistry of tetracycline (I, cf. ref. 9), model studies clearly indicate that the only reasonable structure which will account for all of the facts is that depicted in structure III, which results from formation of an internal hemiketal between the 6-hydroxyl and the newly formed 4-oxo group.

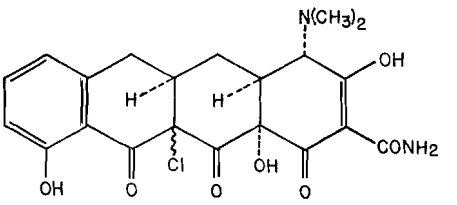
The same reaction has been applied successfully to 11*a*-chlorotetracycline-6,12-hemiketal (II) to provide the 11*a*-chloro-4-oxo analogue (V). Structural assignment is based

[1]*Much of the work described herein has been the subject of a preliminary communication (1). Similar independent studies employing 6-demethyltetracyclines have been reported by a group at Lederle Laboratories (2).*

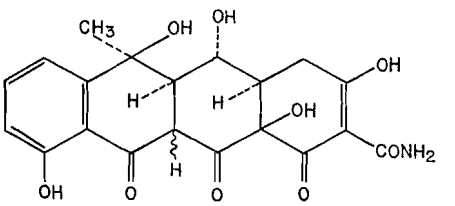


on ultraviolet absorption in methanol – hydrochloric acid (virtually identical with that of the precursor, II), infrared absorption in the carbonyl region (closely analogous to those of III, XV, and XVI), and catalytic reduction to the alcohol (IV) with uptake of 2 equivalents of hydrogen.

The reaction which leads to formation of the 4-oxotetracyclines (III and V) appears to be of a novel type insofar as ketone formation is concerned, although aldehydes have been reported as products in prior studies on the interaction of tertiary amines with N-bromosuccinimide (10) and with hypochlorous acid (11). In the case of the N-bromosuccinimide reaction, initial bromination on carbon adjacent to nitrogen has been indicated (13). We would, therefore, suggest that, in the case of tetracycline, formation of the 4-oxo derivative occurs as shown in Reaction Scheme 1. Esse *et al.* (2), on the basis of additional evidence, have also proposed an imine intermediate in connection with their analogous studies on 6-demethyltetracyclines.

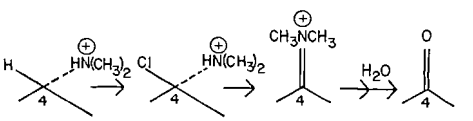


XV



XVI

4-Dedimethylamino-4-oxotetracycline-4,6-hemiketal (III) is readily reduced to the alcohol (IV, e.g. by sodium hydrosulfite, zinc combination, or catalytic hydrogenation). The latter shows the typical ultraviolet chromophores of tetracycline and no infrared carbonyl bands below 6 μ. The alcohol in turn is converted by acid degradation to its 5a,6-anhydro derivative (VI). Preliminary evidence indicates that the same anhydro alcohol is obtained by degradation of the 4,6-hemiketal in anhydrous hydrogen fluoride followed by reduction.



REACTION SCHEME 1.

Reaction of hydrazine and of hydroxylamine with the 4-oxo derivative (III) produces hydrazone (IX) and oxime (VII) respectively. The C,D-β-diketone system in these compounds is enolic, with the now more highly conjugated A-ring ultraviolet chromophores shifted to longer wavelengths. Both compounds are readily reduced to 4-epi-N^4,N^4-didemethyltetracycline (X). Under the conditions which we have employed (sodium hydrosulfite, zinc – acetic acid, or catalytic hydrogenation), it appears that the primary sight of reduction is the N—N or the N—O bond, rather than the C=N double bond. Thus, we have seen no evidence of intermediate hydrazines or hydroxylamines in these reactions. Furthermore, a major by-product in the case of sodium hydrosulfite reduction of VII or IX in aqueous solvents is the 4-hydroxy derivative (IV). Since there is no apparent hydrolysis in the absence of the reducing agent, it is proposed that there is initial reduction to an imine, part of which is reduced to amine and part of which is hydrolyzed to ketone (III) and then reduced to alcohol (IV). The amine (X) may also be obtained directly from the 4-ketone by reductive amination.[2]

[2]*Esse et al. (2) have reported extensively on reductive amination of the 6-demethyl analogue of III. They employed a variety of primary amines, although they did not describe use of ammonia. They have also assigned the 4-epi configuration to their alkylamino products and have shown them to contain the normal more active epimer as a minor component.*

Assignment of stereochemistry at C-4*a* in the 4-amino derivative (X) and its precursors is based on smooth conversion of X to tetracycline methyl betaine (XII) by reaction with methyl iodide, in the presence of excess propylene oxide as an acid scavenger.[3] Tentative assignment of stereochemistry at C-4 was earlier (1) deduced from the short wavelength ultraviolet absorption (almost identical with that of 4-epitetracycline) and from the presence of a minor more antibacterial substance in all preparations of the amine.[2] Unequivocal assignment of stereochemistry at C-4 in compound X derives from degradation studies in hot aqueous bicarbonate, which followed earlier studies by Korst and Butler (12) using an N^4-demethyltetracycline prepared by an unrelated reaction sequence. Under these conditions, the normal epimer persists, and can be readily separated from the degradation product, which has been characterized as the lactam, XIII. This non-basic compound is isomeric with its precursor and possesses 8-hydroxytetralone—A-ring ultraviolet chromophores and infrared absorption at 5.7 μ. It is degraded in acid to compound XIV, which displays ultraviolet absorption typical of 1,8-dihydroxynaphthalene and A-ring chromophores as well as retention of lactam carbonyl absorption. Formation of the lactam (XIII) from X undoubtedly occurs by transannular attack of the 4-amino group on the 12-carbonyl. Such an interaction requires that the amine be *trans* to C-4*a*-hydrogen (4-epi configuration). The undegraded minor component, which was not isolated in analytical pure form, shows the ultraviolet absorption characteristics of tetracycline, and, like the latter, is epimerized at C-4 in pyridine – acetic acid.

EXPERIMENTAL

4-Oxo-4-dedimethylaminotetracycline-4,6-hemiketal (III)

Tetracycline hydrochloride (10 g) was dissolved in 500 ml of water containing 2 ml of concentrated hydrochloric acid. (Initial experiments without added hydrochloric acid gave somewhat lower yields of crude product contaminated by greater amounts of tetracycline.) To the solution, with stirring at room temperature, was added 7 g of powdered N-chlorosuccinimide. The crude product began to precipitate within a few minutes. It was recovered by filtration, with water wash, after 30 min reaction time. Partial purification of the crude air-dried product (6.7 g) was achieved by distributing it between 150 ml of water and 400 ml of ether. The aqueous phase and five 40-ml water washes of the ether phase were discarded. The ether phase was stripped to dryness, the residue treated with water, and the product recovered by filtration. The yield of partially purified product, suitable for further transformations, was 5.6 g (65% overall).

For analysis, partially purified product (1 g) was dissolved in 4 ml of methanol, treated with Darco G-60, and filtered. The product crystallized as the monopotassium salt upon the addition of 0.40 ml of 5 *N* potassium hydroxide. The yield of material, dried *in vacuo* at 50° for 48 h, was 0.50 g (46%). It showed λ_{max}^{KBr} 5.7 μ; $\lambda_{max}^{MeOH - 0.01\ N\ HCl}$ 268 and 346 mμ (log ϵ 4.40 and 3.72); in MeOH – 0.01 *N* NaOH the compound is very unstable—a rapidly determined spectrum includes $\lambda_{max} \sim 370$ mμ (log $\epsilon \sim 4.4$).

Anal. Calcd. for $C_{20}H_{16}O_9NK \cdot 0.5H_2O$: C, 52.0; H, 3.7; N, 3.0; Cl, 0.0; K, 8.4; H_2O, 1.9. Found: C, 52.0; H, 3.8; N, 3.2; Cl, 0.0; K, 8.2; H_2O, 1.7.

Compcund III shows the typical acid instability of tetracyclines having a hydroxyl group at C-6 and unblocked at C-11*a*. For example, when the potassium salt of III was dissolved in liquid hydrogen fluoride, allowed to stand for 1.5 h, and then evaporated to dryness, it gave a crude product, presumed to be 5*a*,6-anhydro-4-oxotetracycline, possessing the expected ultraviolet spectral properties. The latter, on reduction with sodium hydrosulfite, is indicated by spectral and paper chromatographic evidence to yield the same anhydro–hydroxy derivative (VI) obtained by the alternative reduction–dehydration sequence.

4-Hydroxy-4-dedimethylaminotetracycline (IV)

Crude 4-oxo-4-dedimethylaminotetracycline-4,6-hemiketal (III, 25 g) was dissolved in 300 ml of methanol. A solution of sodium hydrosulfite (10 g) in 200 ml of water was added. The temperature rose from 25 °C to 35 °C during this process. The mixture was stirred for 40 min at room temperature. Water (250 ml) was added and the solution extracted twice with 1.25-l portions of ether. The combined ether layers were

[3]*Under these conditions tetracycline and 4-epitetracycline yield the same betaine. Without the acid scavenger, the reaction of X with methyl iodide ceases with uptake of 1 equivalent of the reagent to produce a mixture which is indicated by paper chromatography to include appreciable amounts of starting material, presumed 4-epi-N^4-demethyltetracycline, and 4-epitetracycline, as well as a minor quantity of tetracycline.*

back washed with five 50-ml portions of water, dried over anhydrous sodium sulfate, filtered, and evaporated to dryness in a rotating evaporator. The residue was treated with ca. 250 ml of water and the amorphous product (19.1 g) recovered by filtration. The product was crystallized from hot ethylene dichloride (recovery in three crops, 10.7 g, 33% overall from tetracycline). It shows λ_{max}^{KBr}, no carbonyl bands below 6 μ; $\lambda_{max}^{MeOH - 0.01\,N\,HCl}$ 257 mμ and 362 mμ (log ϵ 4.42 and 3.68); $\lambda_{max}^{MeOH - 0.01\,N\,NaOH}$ 246, 264, and 378 mμ (log ϵ 4.26, 4.23, and 4.28).

Anal. Calcd. for $C_{20}H_{19}NO_9$: C, 57.6; H, 4.6; N, 3.3. Found: C, 57.3; H, 4.6; N, 3.2.

The same 4-hydroxy compound (IV) is also obtained by sodium hydrosulfite reduction of partially purified, or the potassium salt of, 4-oxo-4-dedimethylaminotetracycline. Alternatively, it may be obtained by zinc dust reduction (e.g. in 50% aqueous acetic acid with excess zinc, with stirring for 30 min at room temperature) or catalytic hydrogenation (e.g. H_2, Pd/C in methanol at atmospheric pressure and room temperature) of either IV or the corresponding 11*a*-chloro derivative (V).

11a-Chloro-4-oxo-4-dedimethylaminotetracycline-4,6-hemiketal (V)

11*a*-Chlorotetracycline-6,12-hemiketal (II, ref. 2, 2.3 g) was slurried in 110 ml water containing 0.7 ml of concentrated hydrochloric acid. Powdered N-chlorosuccinimide (1.6 g) was added and the mixture stirred for 1 h at room temperature. The crude product was isolated by filtration with water wash. It crystallized on trituration with methanol. The yield was 11 g. For analysis, 1 g was dissolved in excess ether and filtered. The filtrate was washed with dilute hydrochloric acid and water, dried over anhydrous sodium sulfate, filtered, and finally stripped to dryness, and the residue triturated with methanol. The material, dried *in vacuo*, showed λ_{max}^{BKr} 5.77 μ; $\lambda_{max}^{MeOH - 0.01\,N\,HCl}$ 258 and 343 mμ (log ϵ 4.42 and 3.68).

Anal. Calcd. for $C_{20}H_{16}O_9NCl$: C, 53.4; H, 3.6; N, 3.1; Cl, 7.9. Found: C, 53.0; H, 3.7; N, 2.8; Cl, 8.0.

5a,6-Anhydro-4-hydroxy-4-dedimethylaminotetracycline (VI)

A solution of 4-hydroxy-4-dedimethylaminotetracycline (V, 0.5 g) was boiled in 5 ml of acetone containing 1.0 g of *p*-toluene sulfonic acid until the volume was reduced to ca. 1.5 ml. The product crystallized when the solution was cooled. It was recovered by filtration and dried *in vacuo* for analysis. It showed $\lambda_{max}^{MeOH - 0.01\,N\,HCl}$ 222, 262, 310, and 423 mμ (log ϵ 4.46, 4.70, 3.68, and 4.01).

Anal. Calcd. for $C_{20}H_{17}O_4N$: C, 60.1; H, 4.3; N, 3.5. Found: C, 59.8; H, 4.5; N, 3.3.

4-Hydroxyimino-4-dedimethylaminotetracycline (VII)

Partially purified 4-oxo-4-dedimethylaminotetracycline-4,6-hemiketal (III, 7.5 g) was dissolved in 30 ml of methanol. Potassium bicarbonate (15 g) and hydroxylamine hydrochloride (3.13 g) were added and the slurry stirred for 15 min. The mixture was filtered. Water (120 ml) was added to the mother liquor and the pH was adjusted to 2.0. The partially precipitated product was extracted into excess ether and recovered by evaporation dryness. The residue was slurried with 100 ml of benzene and the insoluble product (4.7 g, 60%) recovered by filtration. The crude product (2.1 g) was recrystallized by dissolving it in 6.3 ml of toluene and 4.2 ml of isopropyl alcohol, filtering it, adding 5.0 ml toluene to the mother liquor, and allowing the mixture to stand at room temperature overnight. The yield of product recovered by filtration and dried *in vacuo* at 50 °C was 1.0 g (48%). It showed $\lambda_{max}^{MeOH - 0.01\,N\,HCl}$ 222, 276, 308, and 359 mμ (log ϵ 4.25, 4.07, 4.23, and 4.19); $\lambda_{inf}^{MeOH - 0.01\,N\,HCl}$ 237 and 268 mμ (log ϵ 4.17 and 4.06); $\lambda_{max}^{MeOH - 0.01\,N\,NaOH}$ 235, 245, and 373 mμ (log ϵ 4.25, 4.24, and 4.28); $\lambda_{inf}^{MeOH - 0.01\,N\,NaOH}$ 256 and 325 mμ (log ϵ 4.21 and 4.16).

Anal. Calcd. for $C_{20}H_{18}O_9N_2$: C, 55.8; H, 4.2; N, 6.5. Found: C, 55.7; H, 4.4; N, 6.2.

5a,6-Anhydro-4-hydroxyimino-4-dedimethylaminotetracycline (VIII)

4-Hydroxyimino-4-dedimethylaminotetracycline (VII, 500 mg) was dissolved in 3 ml of 4% methanolic hydrogen chloride. The solution was boiled about 3 min and left to crystallize overnight at room temperature. The product (240 mg, 51%) was recovered by filtration with methanol wash and dried *in vacuo* at room temperature. It showed $\lambda_{max}^{MeOH - 0.01\,N\,HCl}$ 225, 263, 302, and 422 mμ (log ϵ 4.48, 4.64, 4.26, and 4.07).

Anal. Calcd. for $C_{20}H_{16}O_8N_2 \cdot 0.5CH_3OH$: C, 57.6; H, 4.2; N, 6.6. Found: C, 57.7; H, 4.1; N, 6.8.

4-Hydrazono-4-dedimethylaminotetracycline (IX)

Partially purified 4-oxotetracycline (III, 11.5 g), dissolved in 80 ml of 95% ethanol, was added to a solution of 1.7 ml of hydrazine hydrate in 80 ml of the same solvent. The mixture, which began to precipitate immediately, was stirred for 1 h and then left to stand overnight. The product was recovered by filtration and the crude air-dried product reslurried in an additional 100 ml of 95% ethanol for 1 h at room temperature. The yield of crystalline product, air-dried at room temperature, was 6.0 g (37%). For analysis, a sample was recrystallized from tetrahydrofuran–water. It showed $\lambda_{max}^{MeOH - 0.01\,N\,HCl}$ 264 and 335 mμ (log ϵ 4.16 and 4.53); $\lambda_{max}^{MeOH - 0.01\,N\,NaOH}$ 261, 323, and 373 mμ (log ϵ 4.33, 4.25, and 4.29).

Anal. Calcd. for $C_{20}H_{19}O_8N_3 \cdot H_2O$: C, 53.6; H, 4.7; N, 9.4. Found: C, 53.8; H, 4.6; N, 9.0.

4-Epi-N^4,N^4-didemethyltetracycline (X)

4-Hydrazono-4-dedimethylaminotetracycline (8.7 g) was dissolved in 350 ml of acetone. Water (260 ml) and then 17.4 g of sodium hydrosulfite were added to the stirred solution. The temperature rose from 25 °C to 32 °C. After it was stirred for an additional 30 min at room temperature, the now heterogeneous mixture was treated with charcoal and filtered through a Supercel pad with 1:1 acetone–water wash. The mother

liquor was stripped of acetone. The pH was adjusted to 4.5 and nonamphoteric by-products, including very considerable presumed 4-hydroxy-4-dedimethylaminotetracycline, were removed from the aqueous phase by thorough extraction with ethyl acetate. The desired product was then extracted into butanol (four times with 100-ml portions). The butanol was dried over anhydrous sodium sulfate, filtered, and evaporated to dryness *in vacuo* in a rotating evaporator. The residue was slurried with and filtered from 33 ml of ethyl acetate. The yield of crude air-dried product was 2.6 g. This crude product was dissolved in 100 ml of dimethylformamide. The solution was clarified and 35 ml of water added to the mother liquor. When the solution was stirred slowly for 2 h, the product crystallized. The yield of vacuum-dried 4-epi-N^4,N^4-didemethyltetracycline, solvated with dimethylformamide, was 2.1 g (20%). It shows $\lambda_{max}^{MeOH - 0.01\ N\ HCl}$ 260 and 360 mμ (log ϵ 4.25 and 4.17); $\lambda_{max}^{MeOH - 0.01\ N\ NaOH}$ 246, 266, and 375 mμ (log ϵ 4.23, 4.22, and 4.25). Paper chromatography – bioautography indicate the product to contain a minor, slightly less polar material which is much more active against *Klebsiella pneumonia*.

Anal. Calcd. for $C_{20}H_{20}O_8N_2 \cdot 1.5(CH_3)_2NCHO$: C, 55.9; H, 5.8; N, 9.3. Found: C, 55.8; H, 5.9; N, 9.2.

The same compound (X) may also be obtained by analogous sodium hydrosulfite reduction of the oxime (VII). Alternatively, the hydrozane and the oxime (3 g) in 50% aqueous acetic acid (100 ml) were reduced by stirring for 30 min at room temperature with 3 g of zinc dust. Excess zinc was removed by filtration and the mother liquor evaporated to low volume. Water (100 ml) was added and the pH adjusted to 7.0 with sodium hydroxide. Crude zinc complex of X (3.9 g) was isolated by filtration and air dried. The product was characterized by ultraviolet absorption, paper chromatography, and conversion to the analytically pure 5*a*,6-anhydro derivative.

Compound X was obtained directly from 4-oxotetracycline-4,6-hemiketal by reductive amination. The partially purified hemiketal (III, 2.5 g) and 1.2 g of magnesium chloride hexahydrate were added to a slurry of platinum oxide (300 mg) prereduced in a mixture of 24 ml of dimethyl formamide and 3 ml of 28% ammonium hydroxide. Approximately 5 mmoles of hydrogen was taken up in 2.5 h at room temperature and atmospheric pressure. The catalyst was removed by filtration and crude product precipitated by addition of the mother liquor to 700 ml of ether that was being stirred. The crude magnesium complex (3.1 g) was characterized by ultraviolet absorption, paper chromatography, and conversion to the 5*a*,6-anhydro derivative.

5a,6-Anhydro-4-epi-N^4,N^4-didemethyltetracycline (XI)

The 5*a*,6-anhydro derivative (XI) was readily obtained from crude zinc complex of compound X (2.5 g) by dissolving in 15 ml of tetrahydrofuran and 1.5 ml of conc. hydrochloric acid. The filtered solution was allowed to stand at room temperature for 8 days. The tetrahydrofuran solvated product, which crystallized slowly, was recovered by filtration and dried *in vacuo* at 50 °C for 48 h. The yields were 0.7 g (30% overall from oxime) and 1.2 g (55% overall from hydrazone). It showed $\lambda_{max}^{MeOH - 0.01\ N\ HCl}$ 223, 272, and 423 mμ (log ϵ 4.45, 4.79, and 3.93); $\lambda_{max}^{MeOH - 0.01\ N\ NaOH}$ 229, 271, 338, and 428 mμ (log ϵ 4.39, 4.57, 3.73, and 4.09).

Anal. Calcd. for $C_{20}H_{18}O_7N_2 \cdot (CH_2)_4O \cdot HCl$: C, 56.8; H, 5.4; N, 5.5; Cl^-, 7.0; NH_2, 3.4. Found: C, 56.7, 56.6; H, 5.4, 5.0; H, 5.6, 5.5; Cl^-, 7.1, 7.1; NH_2, 3.2.

The volatile portion was only 3.7% when the compound was dried overnight *in vacuo* at 100 °C. When the compound was dried for 48 h at 100 °C, the analysis was consistent with loss of only part of the tetrahydrofuran.

Anal. Calcd. for $C_{20}H_{18}O_7N_2 \cdot 0.25(CH_2)_4O \cdot HCl$: C, 55.8; H, 4.7; Cl^-, 8.0. Found: C, 55.6; H, 5.0; Cl^-, 7.5.

Identical product was obtained by analogous acid degradation of either the purified 4-amino compound (X, dimethylformamide solvate) or the crude magnesium complex.

Tetracycline Methyl Betaine (XII)

The 4-amino derivative (X, dimethylformamide solvate, 100 mg) was refluxed overnight in a mixture of 2 ml acetone, 0.5 ml propylene oxide, and 0.5 ml methyl iodide. When the mixture was cooled, pure crystalline N^4-methyltetracycline betaine (70 mg, 79%) was recovered by filtration. The same product is obtained by use of this process with either tetracycline or 4-epitetracycline (87% and 84% yield respectively). They show identical behavior on paper chromatography and identical infrared absorption (KBr); $\lambda_{max}^{MeOH - 0.01\ N\ HCl}$ 271 and 358 mμ (log ϵ 4.24 and 4.17).

Anal. Calcd. for $C_{23}H_{26}O_8N_2 \cdot H_2O$: C, 58.0; H, 5.9; N, 5.9. Found: C, 58.0; H, 6.0; N, 5.6.

Tetracycline methyl betaine has been previously reported as a methanol solvate (4).

Degradation of 4-Epi-N^4,N^4-didemethyltetracycline

A solution of the amino compound (X, dimethylformamide solvate, 2.0 g) in 50 ml of 40% aqueous sodium bicarbonate was heated on a steam bath under nitrogen for 30 min. The solution was cooled to room temperature, transferred to a beaker, layered with 50 ml of ethyl acetate, and carefully acidified to pH 2.0 with 5% hydrochloric acid. The aqueous phase was further extracted with six 50-ml portions of ethyl acetate. After drying over anhydrous sodium sulfate, the combined ethyl acetate extracts were evaporated to dryness *in vacuo* to yield 1.34 g of crude lactam degradation product (XIII). To free the product of traces of the 4-amino derivative (principally the normal epimer), the crude product was taken up in

1.9 l of ether and reevaporated to dryness. The recovery was 1.14 g. For analysis, the lactam was recrystallized from hot acetone–water. The recovery in two crops, dried *in vacuo*, was 0.87 g (51%). It shows $\lambda_{max}^{MeOH - 0.01\ N\ HCl}$ 258 and 335 mμ (log ε 4.38 and 3.73); $\lambda_{max}^{MeOH - 0.01\ N\ NaOH}$ 249, 262, and 335 mμ (log ε 4.22, 4.17, and 3.71); $\lambda_{max}^{dioxane}$ 5.72 and 6.07 μ.

Anal. Calcd. for $C_{20}H_{20}O_8N_2 \cdot 2H_2O$: C, 5.31; H, 5.3; N, 6.2. Found: C, 53.2; H, 5.4; N, 6.5.

In a separate experiment 200 mg of compound X was similarly degraded in bicarbonate. The pH was adjusted to 2.0 and the mixture filtered (insoluble, 10 mg). The mother liquor was extracted with eight 15-ml portions of butanol. The combined butanol extracts were evaporated to dryness. The residue (150 mg) was taken up in 20 ml water and 20 ml of ethyl acetate. The aqueous phase, after three further extractions with ethyl acetate, was freeze dried to yield 21 mg of crude N^4,N^4-didemethyltetracycline which paper chromatography indicated to be virtually free of the 4-epi compound. It showed $\lambda_{max}^{MeOH - 0.01\ N\ HCl}$ 268 and 362 mμ, and was readily epimerized (e.g. in acetic acid – pyridine) to compound X (paper chromatographic evidence).

The lactam (XIII) was degraded to the 1,8-dihydroxynaphthalene derivative (XIV) by refluxing the former (300 mg) in 10 ml of 1:6 conc. hydrochloric acid – methanol. The solution was cooled, clarified, and evaporated to dryness. The residue was taken up in excess ether and the ether washed with several small portions of water, dried over anhydrous sodium sulfate, and evaporated to dryness, and the amorphous product dried *in vacuo* for analysis. The yield of compound XIV was 250 mg (92%). It showed $\lambda_{max}^{MeOH - 0.01\ N\ HCl}$ 232, 257, 310, 325, and 339 mμ (log ε 4.43, 3.94, 3.48, 3.56, and 3.59); $\lambda_{max}^{dioxane}$ 5.73 and 6.05 μ.

Anal. Calcd. for $C_{20}H_{18}O_7N_2 \cdot 0.5H_2O$: C, 59.0; H, 4.7; N, 6.9. Found: C, 59.1; H, 5.1; N, 6.4.

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