# COMMUNICATIONS TO THE EDITOR

Tetracycloxides. I. A New Class of Tetracycline Derivatives

Sir:

We wish to report the synthesis and characterization of a new class of tetracycline derivatives which allows for previously unattainable modifications of the fundamental tetracycline structure.[1] We propose that these novel derivatives, which are characterized by a 4,6-oxygen bridge, be named "tetracycloxides"[2] based on the hypothetical compound I.
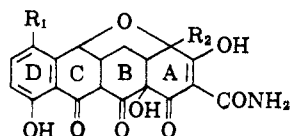
I	R_1 = H, R_2 = H	
II	R_1 = H, R_2 = OH	
III	R_1 = H, R_2 = N(CH_3)_2	
IV	R_1 = Cl, R_2 = OH	
V	R_1 = Cl, R_2 = N(CH_3)_2	

Compound II, the first member of this class encountered, resulted (67% isolated yield) from an attempt to chlorinate 6-demethyltetracycline (VI) with a combination of concentrated hydrochloric acid and sodium chlorate in acetic acid.[3] The same reaction with 6-demethyl-6-deoxytetracycline had given 11a-chloro-6-demethyl-6-deoxytetracycline[4] (50% isolated yield). Since II had the spectral characteristics ($\lambda_{max}^{MeOH, 0.1\ N\ HCl}$ 258 and 338 mμ (log ϵ 4.64 and 3.67); λ_{max}^{KBr} 5.78 μ) of an 11a-chloro substituted tetracycline[5] it was surprising when the elemental analysis revealed, in addition to the loss of dimethylamine, no chlorine to account for the shortening of the B–C–D chromophore. *Anal.* Calcd. for $C_{19}H_{15}NO_9$: C, 56.85; H, 3.77; N, 3.51. Found: C, 56.61; H, 4.02; N, 3.40; Cl, none.

When II was treated with excess methylamine under conditions of reductive amination, the resulting product had incorporated nitrogen and now had a typical tetracycline ultraviolet spectrum with the long wave length maximum at 355 mμ. When the aminated compound was, in turn, reductively methylated (formaldehyde, H$_2$, Pd–C) the product was VI. Coming full circle in this manner made it evident that the product of the reductive amination was 4-dedimethylamino-4-methylamino-6-demethyltetracycline. Since reaction of II with methylamine gave a tetracycline-like prod-

(1) (a) Derivatives of this new class of compounds were first described by R. C. Esse and G. M. Sieger, South African Patent Application 63/4791 (filed Oct. 22, 1963; accepted March 25, 1964). (b) After this communication was submitted, an article appeared describing related work with tetracycline [R. K. Blackwood and C. R. Stephens, *J. Am. Chem. Soc.*, **86**, 2736 (1964)]. (c) See also the accompanying communication: R. C. Esse, J. A. Lowery, C. R. Tamorria, and G. M. Sieger, *ibid.*, **86**, 3875 (1964).

(2) Thus II becomes "4-hydroxytetracycloxide" for which a trivial name is "4-dedimethylamino-4-oxo-6-demethyltetracycline 4,6-hemiketal" while the systematic name is "1,4,4a,5,5a,6,11,11a,12,12a-decahydro-4β,6β-epoxy-3,4α,10,12aα-tetrahydroxy-1,11,12-trioxo-2-naphthacenecarboxamide."

(3) F. Reveridin and P. Crépieux, *Ber.*, **33**, 2503 (1900).

(4) C. R. Stephens, J. J. Beereboom, H. H. Rennhard, P. N. Gordon, K. Murai, R. K. Blackwood, and M. Scach von Wittenau, *J. Am. Chem. Soc.*, **85**, 2643 (1963).

(5) The effect which substitution at C-11a has on the spectral properties of the tetracyclines has been frequently noted [cf. A. Green, R. G. Wilkinson, and J. H. Boothe, *ibid.*, **82**, 3947 (1960), and H. H. Rennhard, R. K. Blackwood, and C. R. Stephens, *ibid.*, **83**, 2775 (1961)].

uct only when reducing conditions were employed, it seemed most likely that C-4 was ketonic. This supposition was supported when II was treated with hydroxylamine to give an oxime ($\lambda_{max}^{MeOH, 0.1\ N\ HCl}$ 309 and 350 mμ (log ϵ 4.32 and 4.19). No λ_{max}^{KBr} between 5 and 6 μ was observed. *Anal.* Found for $C_{19}H_{16}N_2O_9 \cdot CH_3OH$: C, 53.62; H, 4.26; N, 6.38.) Oxime formation was established as having occurred at the C-4 position when the oxime was catalytically reduced in the presence of formaldehyde to give VI. The 4-oxime analog had further significance in that its formation resulted in the removal of the apparent C-11a block of the B–C–D ultraviolet chromophore. It was thus established that reducing conditions were not necessary for the recovery of the long wave length ultraviolet maximum.

The observed chemical transformations (reductive amination, oxime formation) could be explained on the basis of C-4 being ketonic as in structure VII. How-
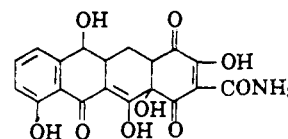
VII

ever, this structure does not account for the apparent block at C-11a. If, however, VII is considered to be in the form of a 4,6-hemiketal it must assume the configuration depicted in VIII. An inspection of molecular
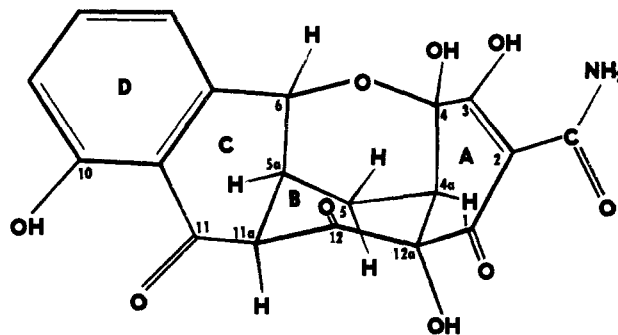
VIII

models of VIII reveals that the 4,6-oxide linkage imposes a nonplanarity on the β-diketone system which prevents enolization of the C-12 ketone. Thus structure VIII, with its ketone (in the form of a hemiketal) at C-4 and its "block" at C-11a, accounts for both the chemical and spectral properties of II.

7-Chloro-6-demethyltetracycline also forms a 4,6-hemiketal (IV) ($\lambda_{max}^{MeOH, 0.1\ N\ HCl}$ 258 and 350 mμ (log ϵ 4.40 and 3.66); λ_{max}^{KBr} 5.79 μ. *Anal.* Found for $C_{19}H_{14}NO_9 \cdot$ dimethylformamide: C, 51.94; H, 3.95; N, 5.58; Cl, 7.09) under the same conditions which lead to II.

It has been found that in addition to sources of positive halogen a variety of reagents can promote tetracycloxide formation. Among them are air, cupric acetate, and mercuric acetate. When a solution of VI in dimethylformamide was treated with 1 mole of mercuric acetate, a new product was detected. It

had the characteristic spectral properties of II but an analysis ($\lambda_{max}^{MeOH,\ 0.1\ N\ HCl}$ 258 and 336 mμ (log ϵ 4.36 and 3.70); λ_{max}^{KBr} 5.83. *Anal.* Found for $C_{21}H_{20}N_2O_8$: C, 58.75; H, 4.86; N, 6.46) revealed that the dimethyl-amino group was still present. Mild acid hydrolysis readily converted it to II. When it was reduced with sodium borohydride under alkaline conditions it gave back VI having the natural configuration at C-4. This product must certainly be 4-dimethylaminotetra-cycloxide (III) and its isolation necessarily sheds light on the sequence of events leading from VI to II. It would appear that the net effect of attack by positive halogen, or the previously noted oxidizing agents, is the loss of hydride ion resulting in the formation of the ternary iminium compound X.

$$H_3C \quad CH_3$$
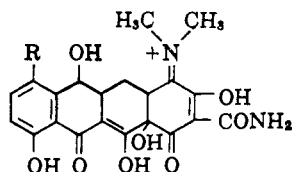
X, R = H or Cl

Compound X would be subject to ready attack by water to give the 4-keto analog, which in turn would undergo hemiketal formation with the C-6 hydroxyl to give the 4-hydroxytetracycloxide. Alternatively, under essentially anhydrous conditions X would simply undergo nucleophilic attack by C-6 hydroxyl to give the 4-dimethylaminotetracycloxide.[6]

Although the stereochemistry of 7-chlorotetracycline has now been completely defined by X-ray crystal-lography,[7] III does provide chemical confirmation for the relative configurations of four of the five asymmetric centers in 6-demethyltetracycline. The configuration at carbons 4a,[8] 5a, 6, and 12a is rigidly defined since the 4,6-oxide bridge can form only when the relative stereochemistry is as shown in VIII.

(6) For a discussion of the formation and reactions of ternary iminium compounds see, for example: N. J. Leonard, A. S. Hay, R. W. Fulmer, and W. V. Gash, *J. Am. Chem. Soc.*, **77**, 439 (1955); N. J. Leonard and A. S. Hay, *ibid.*, **78**, 1984 (1956).

(7) J. Donohue, J. D. Dunitz, K. N. Trueblood, and M. S. Webster, *ibid.*, **85**, 851 (1963).

(8) There is some slight ambiguity concerning C-4a since epimerization at this site could conceivably occur *via* the nonprotonated (enamine) form of X.

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## Tetracycloxides. II. Transformations at the C-4 Position

Sir:

It was noted in the accompanying report[1] that 4-hydroxytetracycloxide can be reductively aminated catalytically with methylamine to give 4-dedimethyl-amino-4-methylamino-6-demethyltetracycline (II) and that this product can, in turn, be reductively methyl-ated with formaldehyde to yield 6-demethyltetra-cycline (VIII). The reductive amination step has been extended to include the use of ammonia and a number of simple aliphatic primary amines. The re-

(1) R. C. Esse, J. A. Lowery, C. R. Tamorria, and G. M. Sieger, *J. Am. Chem. Soc.*, **86**, 3874 (1964).
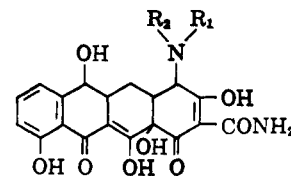
I-XII

sulting derivatives are summarized in Table I. The reductive aminations were carried out at room tem-perature at pressures of 50 lb. or less, with 10% pal-ladium on carbon as the catalyst. Generally, 10 equiv. of amine was used. The large excess of amine served two purposes; it afforded the alkaline conditions necessary for the success of the reaction and it mini-mized the formation of 4-dedimethylamino-4-hydroxy-6-demethyltetracycline.[2] These reactions were rapid, being complete within 20 min. Even so, there was a considerable loss in side reactions which stems from the great alkaline instability of 4-hydroxytetracycloxide. Rapid handling of the reaction solution prior to commencing hydrogenation was essential to minimize losses. The yields of crystalline product isolated ranged from 20 to 40%.

Three factors strongly suggest that the products of Table I have mainly the 4-*epi* configuration: (1) the A ring ultraviolet chromophore of these products has its maximum at *ca.* 255 mμ which is characteristic of 4-*epi*-tetracyclines[3]; (2) reductive methylation of II gives mainly the known 4-*epi*-6-demethyltetracycline[4]; and (3) column chromatography of II has permitted the isolation of a minor component which has the ultra-violet spectrum, relative polarity, and increased bio-logical activity (7-fold) which would be expected for the "natural" C-4 epimer of II. Attempts to epi-merize the products of Table I in glacial acetic acid or by formation of a calcium chelate as described by Nose-worthy[5] failed.

A number of the derivatives in Table I have been reductively alkylated catalytically. Of the new com-pounds formed, those which have been isolated in pure form are listed in Table II. The reductive alkylations were run overnight at 50 lb. pressure and were best accomplished at somewhat elevated temperatures (50–60°). The crude products from the reductive alkylation were subjected to the epimerizing condi-tions[5] which failed with the 4-amino and 4-monoalkyl-amino analogs. In contrast, the 4-dialkylamino ana-logs were epimerized completely. The compounds reported in Table II have the "natural" configura-tion at C-4.

The antimicrobial potencies of the N-demethyl analogs of Table I are very low in comparison with the fermentation-derived tetracyclines. This is to be expected since they have the 4-*epi* configuration but, beyond that, a small amount of the "natural" epimer of II was isolated and it assayed[6] at only 7% of tetra-

(2) The 4-hydroxy analog has not been fully characterized, but we have little doubt as to its identity.

(3) J. R. D. McCormick, S. M. Fox, L. L. Smith, B. A. Bitler, G. Reichen-thal, V. E. Origoni, W. H. Muller, R. Winterbottom, and A. P. Doerschuk, *J. Am. Chem. Soc.*, **79**, 2849 (1957).

(4) J. R. D. McCormick, N. O. Sjolander, U. Hirsch, E. R. Jensen, and A. P. Doerschuk, *ibid.*, **79**, 4561 (1957).

(5) M. M. Noseworthy, U. S. Patent 3,009,956 (Nov. 21, 1961).

(6) Activities were measured turbidimetrically against *Staphylococcus aureus* by the method of E. Pelcak and A. Dornbush, *Ann. N. Y. Acad. Sci.*, **51**, 218 (1948).