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6-Deoxytetracyclines. IV.[1,2] Preparation, C-6 Stereochemistry, and Reactions

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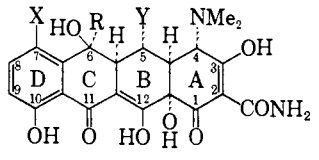
This paper describes methods for preparation of 6-deoxytetracyclines and some side reactions noted. Stereochemistry at C-6 is assigned in pertinent cases. A number of acid-catalyzed reactions, made possible by the acid stability of the 6-deoxy compounds, are presented. Some structure-activity data are included.

The C-6 benzylic hydroxyl function present in the molecule of the fermentation derived tetracyclines (I)[3] has historically presented a center of instability

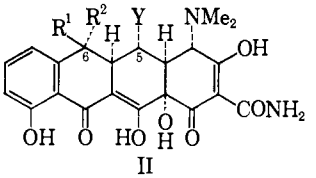


I

	X	R	Y
Ia	H	CH_3	H
Ib	H	CH_3	OH
Ic	H	H	H
Id	Cl	H	H
Ie	Cl	CH_3	H

which, for several years, seriously limited the scope of chemical reactions applicable to this important class of antibiotics.[2b] However, the stability conferred upon the molecule by removal of the labile C-6 hydroxyl and the observation that this group is not essential for antimicrobial activity[1a] has stimulated renewed interest in derivative and synthesis[4] studies in the tetracycline field. This paper describes the methods we have used to prepare 6-deoxytetracyclines and their derivatives, including some side reactions noted. In cases where C-6 is asymmetric, stereochemistry is assigned. A variety of acid-catalyzed transformations, new to the tetracycline series, are also described.

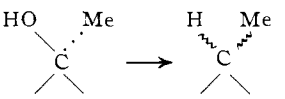
Preparation and C-6 Stereochemistry.—6-Deoxytetracyclines were originally isolated from the mixture of products obtained by hydrogenolysis of the parent antibiotics (I) in the presence of acid and a noble metal catalyst such as palladium. In the cases involving 7-chlorotetracyclines (Id, Ie) the 7-chlorine atom is usually hydrogenolyzed also.[5] Since the acid catalyst is essential, concurrent 5a,6-dehydration[3b] initiates a series of competing side reactions in all cases. Tetracyclines which do not contain a C-6 methyl group, *e.g.*, 6-demethyltetracycline (Ic) and 7-chloro-6-demethyltetracycline (Id), undergo reductive removal of the secondary benzylic hydroxyl in satisfactory yield (30–40%). The product formed in these cases, 6-demethyl-6-deoxytetracycline (IIa), is of particular interest in that it represents the simplest member of the



II

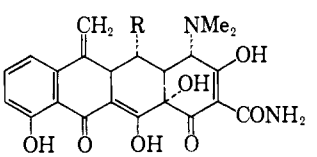
	R^1	R^2	Y
IIa	H	H	H
IIb	CH_3	H	H
IIc	CH_3	H	OH
IId	H	CH_3	H
IIe	H	CH_3	OH

series reported to show a full complement of antimicrobial activity.[2a,4] It will be noted that compound IIa has lost asymmetry at the reaction center (C-6). Because side reactions are a more serious complication, tetracyclines containing a C-6 methyl group (Ia, Ib, Ie) give, on hydrogenolysis, less favorable yields (5–20%) of the 6-deoxy derivatives (IIb, IIc). This is especially true in the case of tetracycline (Ia). Furthermore, the transformation at C-6, *i.e.*



involves an asymmetric center, and, though only one 6-deoxy epimer is recoverabe from either Ia or Ib, it was not apparent from our early hydrogenolysis experiments what configurational relationships prevailed. Light was subsequently shed on the problem of C-6 stereochemistry by the synthetic studies of Muxfeldt,[6] who concluded that inversion had occurred in the deoxygenation of 7-chlorotetracycline,[5] and by our own extended studies[1c] detailed herein.

The availability of the 6-methylenetetracyclines[7]



IIIa, R=OH
IIIb, R=H

(III) made possible a solution to the preparative and stereochemical problems in the 6-methyl-6-deoxy series. 6-Methylene-5-hydroxytetracycline (IIIa) was particularly informative in that on hydrogenation it furnished, in good yield, an approximately equimolar, readily separable mixture of the two C-6 epimers of 6-deoxy-5-hydroxytetracycline. The least active of these which we now call the β-epimer[11b] (IIc), was found to be identical with the compound obtained by direct hydrogenolysis of 5-hydroxytetracycline. The α-epimer[11b] (IIe) was observed to be appreciably more active against bacteria than the parent 5-hydroxytetracycline (Ib).

(1) For preliminary communications on this material see: (a) C. R Stephens, K. Murai, H. H. Rennhard, L. H. Conover, and K. J. Burnings, *J. Am. Chem. Soc.*, **80**, 5324 (1958); (b) J. J. Beereboom, J. J. Ursprung, H. H. Rennhard, and C. R. Stephens, *ibid.*, **82**, 1003 (1960); (c) M. Schach von Wittenau, J. J. Beereboom, R. K. Blackwood, and C. R. Stephens, *ibid.*, **84**, 2645 (1962). *Cf.* also ref. 7 and 8.

(2) Several related, independent studies in this area have also appeared. *Cf.* (a) J. R. D. McCormick, E. R. Jensen, P. A. Miller, and A. P. Doerschuk, *J. Am. Chem. Soc.*, **82**, 3381 (1960); (b) J. J. Hlavka, A. Schneller, H. Krazinski, and J. H. Boothe, *ibid.*, **84**, 1426 (1962); (c) J. Petisi, J. L. Spencer, J. J. Hlavka, and J. H. Boothe, *J. Med. Pharm. Chem.*, **5**, 538 (1962); (d) J. J. Hlavka, H. Krazinski, and J. H. Boothe, *J. Org. Chem.*, **27**, 3674 (1962).

(3) *Cf.* (a) 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, *J. Am. Chem. Soc.*, **75**, 5457 (1953); (b) 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); (c) J. R. D. McCormick, N. O. Sjolander, V. Hirsch, E. R. Jensen, and A. P. Doerschuk, *ibid.*, **79**, 4561 (1957); *cf.* also footnote 9.

(4) A culmination of these efforts has been the recent total synthesis of 6-demethyl-6-deoxytetracycline (IIa); *cf.* L. H. Conover, K. Butler, J. D. Johnston, J. J. Korst, and R. B. Woodward, *J. Am. Chem. Soc.*, **84**, 3222 (1962).

(5) Under special conditions, *e.g.*, with rhodium catalyst, this chlorine is partially retained during 6-deoxygenation. *Cf.* J. R. D. McCormick and E. R. Jensen, U. S. Patent 3,019,260, January 30, 1962.

(6) H. Muxfeldt, *Angew. Chem.*, **74**, 443 (1962); H. Muxfeldt, W. Rogalski, and K. Striegler, *Ber.*, **95**, 2581 (1962).

(7) R. K. Blackwood, J. J. Beereboom, H. H. Rennhard, M. Schach von Wittenau, and C. R. Stephens, *J. Am. Chem. Soc.*, **83**, 2773 (1961). "6-Methylenetetracycline" is a contraction of the name 6-deoxy-6-demethyl-6-methylenetetracycline. Alternative nomenclature would be 6,13-anhydrotetracycline (*cf.* footnote 1 of ref. 7). The part of this communication concerned with 6-deoxytetracyclines is detailed in the present paper.

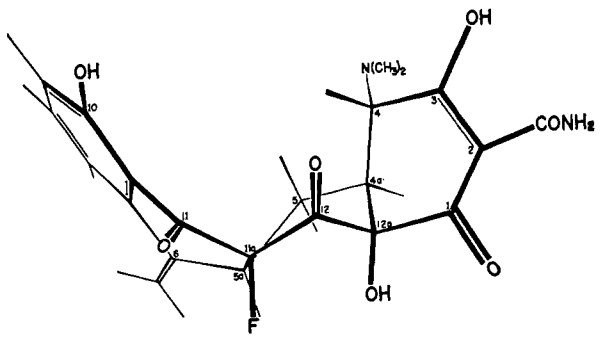
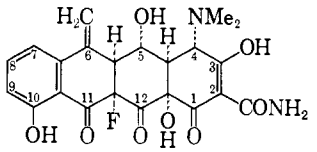


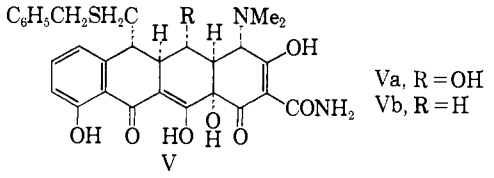
Figure 1.

Chemical information on the configuration of the C-6 methyl in the β-series was forthcoming when we observed that catalytic hydrogenation of 11α-fluoro-6-methylene-5-hydroxytetracycline[7] (IV) yields predominantly the β-epimer IIc. The skeletal configuration of IV is established.[7-9] Model studies indicate clearly that IV is best represented by the conformation shown in Fig. 1. It can be seen that the curvature of the molecule is such that the methylene function can approach the catalyst most readily from the side of the C-5α hydrogen. Attack of hydrogen in this manner would result in the β-configuration at C-6, with the C-6 methyl and C-5a hydrogen *trans* (IIc).



IV

Further confirmation of the C-6 stereochemistry was obtained by an alternative, stereospecific synthesis of the α-epimer IIe. It has been observed that benzylmercaptan will add to the methylene group of III through a free-radical mechanism to give the adduct V.[10]



Va, R = OH
Vb, R = H

V

Only one C-6 epimer is obtained. From model studies we would expect the more stable form to be that with the large group at C-6 equatorial, or with the α-configuration as shown. Raney nickel desulfurization of Va leads to pure α-6-deoxy-5-hydroxytetracycline (IIe).

Catalytic hydrogenation of 6-methylenetetracycline[7] (IIIb) results in a substantial yield of the same deoxy compound (IIb) obtainable directly from tetracycline, together with a smaller quantity of a new, more highly active epimer (IId). Considerable amounts of anhydrotetracycline[3b] and its reduction products (*vide infra*) are also formed in this reaction. Application of the mercaptan addition–Raney nickel desulfurization sequence to 6-methylenetetracycline yields the more active epimer, α-6-deoxytetracycline (IId), as the only isolated product. Configurational assignments rest on analogy to the previous case.

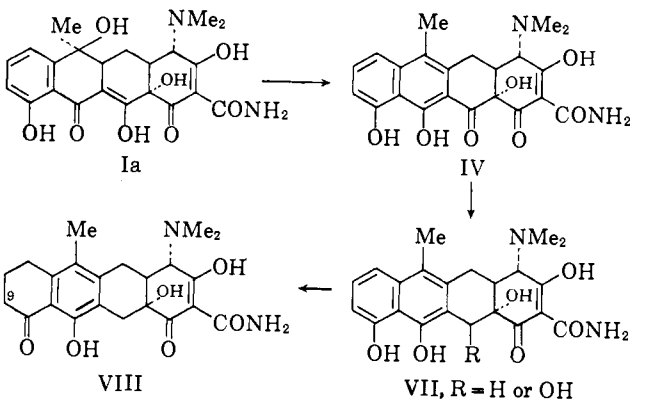
In vitro biological assay data on the 6-deoxy epimers, as well as on the 6-demethyl analog IIa, are presented in Table I. The reduced activity in the β-series may well be the result of conformational distortion.[11a,11b]

TABLE I

Compound, tetracycline	Bioassay[a] *vs. K. pneumoniae*[b]
6-Demethyl-6-deoxy- (IIa)	900
β-6-Deoxy- (IIb)	500
β-6-Deoxy-5-hydroxy- (IIc)	400
α-6-Deoxy- (IId)	700
α-6-Deoxy-5-hydroxy- (IIe)	1400

[a] Expressed in oxytetracycline units/mg. *Cf.* R. C. Kersey, *J. Am. Pharm. Assoc.*, **39**, 252 (1950). In this assay 5-hydroxytetracycline is taken as the standard at 1000 units/mg. We are indebted to Mr. J. J. Smith and his associates for these measurements. [b] Similar relative activities have been noted with other microorganisms.

Side Reactions.—As noted earlier, the most troublesome side reactions in the formation of 6-deoxytetracyclines involve an initial dehydration to 5a,6-anhydrotetracyclines.[3b] This dehydration is catalyzed not only by hydrogen ions but also by noble metals and hydrogen.[12] Once formed, the anhydrotetracycline undergoes hydrogenation at a rate which is frequently comparable to C-6 deoxygenation. The nature of the by-products formed was worked out in some detail for the case of tetracycline, perhaps the worst offender in the magnitude of side reactions. Low hydrogen pressure and high acidity seem to favor the dehydration over deoxygenation. Under these conditions approximately three moles of hydrogen are consumed. By interrupting the reaction at appropriate intervals it was established that the predominant course of the side reaction sequence proceeded as



Ia IV VIII VII, R = H or OH

Compound VIII has been obtained in good yield from both tetracycline and anhydrotetracycline. The structural assignment shown for VIII is based on its mode of

(8) H. H. Rennhard, R. K. Blackwood, and C. R. Stephens, *J. Am. Chem. Soc.*, **83**, 2774 (1961). Details of this communication concerned with 6-deoxytetracyclines are detailed in the present paper.

(9) (a) *Cf.* V. N. Dobrynin, A. I. Gurevich, M. G. Karapetyan, M. N. Kolosov, and M. M. Shemyakin, *Tetrahedron Letters*, **20**, 901 (1962), for the assignment of absolute configuration to the tetracyclines and for a review of chemical and early X-ray investigations which have contributed to the establishment of relative configuration. (b) For X-ray studies on the stereochemistry of 5-hydroxytetracycline *cf.* Y. Takeuchi and M. J. Buerger, *Proc. Natl. Acad. Sci. U. S.*, **46**, 1366 (1960). Since completing this manuscript it has come to our attention that the C-5 stereochemistry of Takeuchi and Buerger has been questioned by J. Donohue, J. D. Dunitz, K. N. Trueblood, and M. S. Webster, *J. Am. Chem. Soc.*, **85**, 851 (1963).

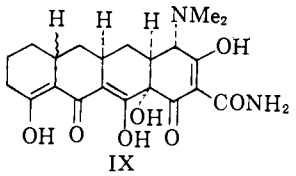
(10) R. K. Blackwood and C. R. Stephens, *ibid.*, **84**, 4157 (1962).

(11) (a) Several authors (*cf.* ref. 2a, 2b) have apparently overlooked potential C-6 stereochemical effects in drawing structure–activity correlations in the 6-deoxytetracycline series. (b) Prof. Muxfeldt (*cf.* ref. 6) employs an alternative nomenclature: "6-epi-6-deoxy" for our "β-6-deoxy" and "6-deoxy" for our "α-deoxy."

(12) (a) We are indebted to Dr. L. H. Conover for early observations on these phenomena. (b) In view of the apparent invariable coproduction of anhydro compounds with 6-deoxytetracyclines by hydrogenolysis it seems attractive to speculate that both may arise from a common intermediate (radical or ion) *on the catalyst surface*.

formation, acidity constants, ultraviolet absorption (that of a substituted 8-hydroxytetralone[13]), n.m.r. spectrum (no aromatic hydrogen evident), and the qualitative observations that (1) the ultraviolet chromophore of VIII is stable to boiling alkali, indicating absence of the 11,12-β-diketone system, and (2) the compound (as evidenced by characteristic ultraviolet chromophore changes) undergoes ready condensation with aldehydes to form benzal type derivatives, indicating the presence of an active methylene group (C-9). The gross structure of the intermediate 1,8-dihydroxynaphthalene (VII) was apparent from its very characteristic ultraviolet absorption[13] and great sensitivity to oxygen. The sequence by which the oxygen atom is cleaved from the C-12 position was not investigated in our laboratory, although correlation of our results with a later report of McCormick and co-workers[2a] suggests that, under certain conditions, this occurs after the hydrogenolysis of ring D. 5-Hydroxytetracycline (Ib) and the 6-demethyltetracyclines (Ic, Id) undergo side reactions similar to those above. In the case of 5-hydroxytetracycline (Ib) the sequence is further complicated by the known rapid rearrangement of the intermediate anhydrooxytetracycline to the apoterramycins,[3a] which then undergo hydrogenation. 6-Methylenetetracyclines also exhibit the anhydro byproduct sequence but to a lesser extent.

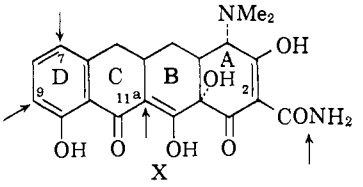
An interesting impurity is frequently observed to be present in 6-demethyl-6-deoxytetracycline (IIa), even after repeated crystallizations. Though present in small amount (*ca.* 5%) the substance is readily detected by a characteristic blue fluorescence (in contrast to the normal yellow-orange of a tetracycline type) which it exhibits on a paper strip chromatogram under ultraviolet light. The compound was thus called "Blue X." "Blue X" could not be obtained from an anhydro intermediate but could be prepared in substantial yield, together with other products, by hydrogenation of 7-chloro-6-demethyltetracycline (Id) over a rhodium catalyst at high pressure.[14] When the compound was isolated (by Craig countercurrent distribution) its composition was noted to be that of a tetrahydro derivative of IIa. Ultraviolet absorption, n.m.r., and pK_a studies lead us to advance expression IX, or a tautomer, as a likely structure for "Blue X."



IX

The substance is devoid of antimicrobial activity.

Reactions.—The 6-deoxytetracyclines undergo many typical reactions previously observed in the tetracycline series; these include nitrile formation,[3a,3b] quaternization,[15] reductive removal of the C-4 dimethylamine group[16] or of the 12-hydroxyl function,[17] C-4 epimerization,[18] etc. In addition, the enhanced acid stability of these substances has rendered possible many acid-catalyzed transformations never before accomplished in the tetracycline series. Illustrative substitution reactions are the transformations we have carried out on 6-demethyl-6-deoxytetracycline. Centers where substituents have been introduced are illustrated by structure X. Products

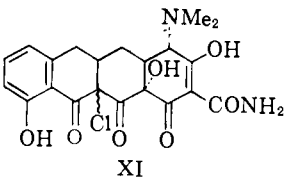


X

prepared by electrophilic substitution include aromatic halo, nitro, and sulfo compounds, as well as 11a-halo compounds. Other D-ring substituted products have been obtained by reduction of nitro to amino groups and subsequent diazotization and Sandmeyer type reactions.[19] Alkyl substitution of the carboxamide has been accomplished through a Ritter reaction[20] on the nitrile.

Electrophilic attack at the C-11a enol position is a usual competitive reaction to aromatic substitution. This is best illustrated by halogenation, where initial attack occurs first at C-11a, then on the aromatic ring. When the entering halogen is bromine or iodine the initially formed 11a-halo compounds are quite labile and may themselves act as active halogenating agents. Hlavka and co-workers[2b] have proposed that strong acid stabilizes the C-11a position of 6-deoxytetracyclines against attack by an electrophilic halogen. This conclusion, based on their results in bromination and iodination experiments, is not tenable when applied to chlorination reactions. We prefer the interpretation that the role of strong acid is to govern the point of equilibrium in cases where only one mole of reagent is employed in iodinations or brominations. Thus, when the entering halogen is chlorine[7] or fluorine (from perchloryl fluoride–alkali)[8,21] the 11a-halo compounds are stable, readily isolated substances. They do, however, undergo relatively facile reductive dehalogenation. These compounds are thus quite useful as C-11a protected intermediates for further reactions.

Structures of the aromatic and enolic halogen substitution products of 6-demethyl-6-deoxytetracycline (II) were readily deduced from chlorination studies using N-chlorosuccinimide. When the compound was chlorinated under mild conditions with one mole of reagent there was obtained a stable monochloro compound whose ultraviolet and infrared absorption properties (*i.e.*, 8-hydroxytetralone and tetracycline A-ring ultraviolet chromophores and a 5.7 μ infrared peak) clearly identify it to be the 11a-chloro compound XI. Mild reduction (sodium hydrosulfite, zinc combinations,



XI

acetone–HI, etc.) of XI readily regenerated starting material.[22] Further N-chlorosuccinimide treatment of XI under more vigorous conditions, then mild reduction, gave a mixture of two compounds, each monochlori-

(13) *Cf.* ref. 3a.

(14) We are indebted to Mr. T. Morris for this experimental observation and other interesting high pressure studies.

(15) J. H. Boothe, G. E. Bonvicino, C. W. Waller, J. P. Petisi, R. W. Wilkinson, and R. B. Broschard, *J. Am. Chem. Soc.*, **80**, 1654 (1958).

(16) C. R. Stephens, U. S. Patent 2,786,077, March 19, 1957.

(17) R. K. Blackwood, H. H. Rennhard, and C. R. Stephens, *J. Am. Chem. Soc.*, **82**, 5194 (1960).

(18) 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).

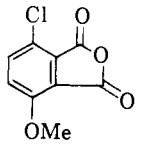
(19) *Cf.* ref. 2b for additional examples of these.

(20) J. J. Ritter and P. Minieri, *J. Am. Chem. Soc.*, **70**, 4045 (1948).

(21) *Cf.* C. E. Inman, R. E. Oesterling, and E. A. Tyczkowski, *ibid.*, **80**, 6533 (1958).

(22) This reductive reconversion is also accomplished enzymatically in several animal species including man.

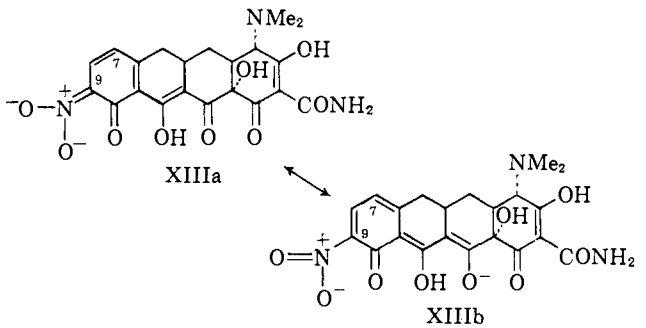
nated in the D-ring. The chloro compound formed in greatest amount was converted to 3-chloro-6-methoxyphthalic anhydride[23] (XII) by an exhaustive methylation–oxidation sequence as previously applied[23] to



XII

7-chlorotetracycline (Ie). Thus the major product is the 7-chloro compound and the minor product the 9-chloro (XIVa).

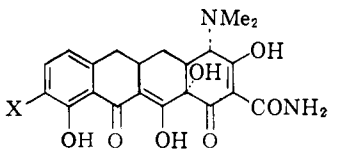
Aromatic nitro derivatives, and the amines derived therefrom, were related structurally to the C-7 and C-9 chloro analogs through conversion of the amines to the chloro compounds *via* the Sandmeyer reaction. A most convenient diagnostic test[1b] for differentiating a 9-nitro-6-deoxytetracycline from a 7-nitro analog[24] is the strikingly greater shift in ultraviolet absorption noted with the 9-nitro analogs in comparing terminal absorption peaks in acidic and alkaline solution. For example, 9-nitro-6-demethyl-6-deoxytetracycline has a terminal absorption maximum at 424 mμ in 0.01 *N* methanolic sodium hydroxide, whereas the 7-nitro analog has a maximum at 385 mμ under similar conditions.[1b] In acidic solutions the 9- and 7-isomers show much more similar absorption (terminal λ_{max} 360 and and 354 mμ, respectively). This phenomenon can be explained on the basis of the more extended conjugation possible in resonance forms in the case of the 9-nitro phenolate anion (*e.g.*, XIIIa, XIIIb). A similar though less pronounced effect is noted in comparing



XIIIa XIIIb

absorption of the *o*- and *p*-nitrophenols.

The Sandmeyer reaction is quite useful in the 6-deoxytetracycline series for the preparation of pure 9-

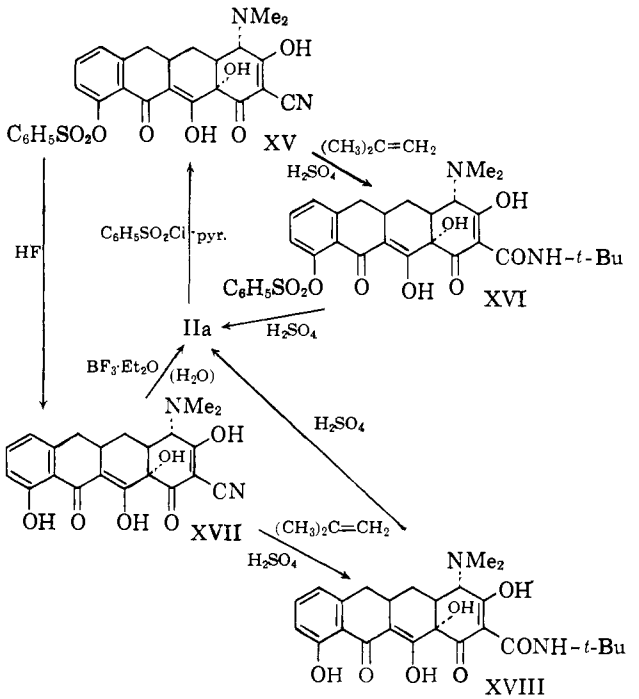


XIVa, X = Cl
b, X = Br
c, X = I

halo compounds (XIV) from 9-amino compounds. As is typical of this type of reaction, particularly favorable yields are obtained when the halogen is iodine. This has been of value as a convenient preparation of 9-I^{131}-6-demethyl-6-deoxytetracycline for localization studies in cancer,[25] since the radioactive atom may be introduced directly from commercially available KI^{131}.

The 9-halo(iodo, chloro, or bromo)-6-demethyl-6-deoxytetracyclines have been consistently less active than the unsubstituted compound IIa in *in vitro* antimicrobial assays (*K. pneumoniae*). This is to be contrasted with equal or enhanced activity noted with the 7-halo compounds.[1b,ab] An even more dramatic difference is noted in comparing activities of 7- and 9-nitro analogs: A 7-nitro substituent strongly enhances *in vitro* activity, whereas a 9-nitro group results in over 90% inactivation.[1b,2b] It is speculated that a strongly electronegative function at C-9 interferes, perhaps by hydrogen bonding, with an essential role of the adjacent C-10 phenolic hydroxyl in some enzyme inhibition reaction. It is interesting to note that amino substituents (electron donating) at either the 7- or 9-position have very little effect on *in vitro* activity *vs. K. pneumoniae*.

Nitrile analogs and 10-benzenesulfonyl nitriles[26] are versatile intermediates in the 6-deoxy series. Thus in addition to their use in preparing N-alkyl amide derivatives, *via* the Ritter reaction,[20,27] the 6-deoxy nitriles may also be reconverted conveniently to the parent amides by several routes involving strong acid treatment. This reconversion opens up another potential relay point for total synthesis efforts. Nitrile transformations effected with 6-demethyl-6-deoxytetracycline (IIa) are illustrated below.



N-*t*-Butyl-6-demethyl-6-deoxytetracycline (XVIII) represents the first example of a simple alkylated amide derivative of a fully active tetracycline. It is of considerable interest from a structure–activity viewpoint because its *in vitro* antibacterial spectrum is much more

(23) S. Kushner, J. H. Boothe, J. Morton, J. Petisi, and J. H. Williams, *J. Am. Chem. Soc.*, **74**, 3710 (1952).

(24) (a) The nitration of 6-deoxytetracycline has recently been discussed in detail by Petisi, Spencer, Hlavka, and Boothe (ref. 2c). These workers used 7-tritio-6-deoxytetracyclines in structural studies. Hlavka and coworkers (ref. 2b) have also used 7-tritio compounds in structural work on 7-halo-6-deoxytetracyclines. (b) Our nitration studies (ref. 1b) involved procedures very similar to those described in ref. 2c. We are indebted to Dr. J. J. Ursprung for these studies.

(25) A. L. Dunn, private communication; *cf.* J. J. Hlavka and D. A. Buyske, *Nature*, **186**, 1064 (1960).

(26) These derivatives are formed by reactions completely analogous to those used earlier with the C-6 hydroxy analogs, *cf.* ref. 3a, 3b, and the Experimental section of this paper.

(27) The Ritter reaction had previously been applied successfully to 5a,6-anhydrotetracyclinonitriles; *cf.* C. R. Stephens, U. S. Patent 3,028,409, April 3, 1962.

narrow in range than that of IIa, covering only Gram positive organisms.[28]

Experimental

Melting points, where indicated, were taken in Pyrex capillary tubes using a calibrated thermometer. Most tetracycline-like substances show decomposition points which vary considerably with rate of heating and are thus of limited significance. Unusually stable solvates with a variety of solvents are common phenomena in this series.[3a,3b] Paper chromatography is a valuable tool for judging the purity of tetracycline derivatives. Several statements regarding percentage purity, reaction course, etc., are based on paper chromatographic analyses[29]—both by qualitative comparisons and by semiquantitative methods involving elution from the paper followed by ultraviolet absorption measurements. Similar techniques have been described by Selzer and Wright[30] for tetracycline and its C-4 epimer.

Typical paper chromatography systems used in this work are illustrated by systems 1 to 3: system 1, mobile phase: nitromethane(200)–toluene(100)–1-butanol(50)–pyridine(30) (volume ratios); immobile phase: pH 3.5 McIlvaine's buffer; sample applied to wet paper. System 2, mobile phase: ethyl acetate(400)–nitromethane(250)–chloroform(70); immobile phase: McIlvaine's pH 4.2 buffer; sample applied to wet paper. System 3 (preferred for quantitative chromatography), mobile phase: the chloroform layer of a 2:1:1 mixture of chloroform–methanol–water; immobile phase: dry paper after saturating with pH 3.4 McIlvaine's buffer then impregnate with 25% water in acetone; apply sample to wet paper.

6-Demethyl-6-deoxytetracycline (IIa).[31]—7-Chloro-6-demethyltetracycline hydrochloride[3c] (15 g.) was stirred in an autoclave at 40° in methanol (150 ml.) solution with 5% palladium-on-charcoal catalyst (5 g.) and hydrogen at an initial pressure of 1000 p.s.i. After 6 hr. the solution was freed of catalyst, treated with 2.5 ml. of concentrated sulfuric acid, and allowed to crystallize with stirring, first at 25° (2 hr.) then at 5° (2 hr.). The resultant slurry was filtered, washed with cold methanol, then with cold acetone. There was obtained 4.6 g. (30% yield) of 6-demethyl-6-deoxytetracycline sulfate, contaminated with small amounts of 6-demethylanhydrotetracycline,[3c] C-4 epi compounds, and "Blue X" (IX). The sulfate (3 g.) was converted to the base by dissolving in water at pH 9 then lowering the pH to 7.5. This treatment effectively removed anhydro-type impurities. The base (2.5 g.) so formed was slurried in 30 ml. of water and adjusted with concd. HCl to pH 1.6. The resulting acidic slurry was warmed until solution occurred; the solution was then filtered and treated with an additional 1 ml. of cond. HCl. 6-Demethyl-6-deoxytetracycline hydrochloride of approximately 95% purity (m.p. *ca.* 220° dec.) separated as pale yellow needles. For analysis, these were dried *in vacuo* at 100° to constant weight.

Anal. Calcd. for $C_{21}H_{23}N_2O_7Cl$: C, 55.94; H, 5.14; N, 6.21. Found: C, 55.78; H, 5.28; N, 6.09.

As previously noted, material obtained as above still contains about 5% of "Blue X" by-product (proposed structure IX). A completely pure product may be prepared by the following procedure: A two-phase system consisting of methanol (10)–water (10)–carbon tetrachloride (5)–chloroform (15) (volume proportions) was prepared. The impure hydrochloride (10 g.) was dissolved in 200 ml. of each phase; the pH of the upper phase was adjusted to 7.0 with Na_2HPO_4 after the dissolution; the resulting solution was placed in the first 20 tubes of a 200-tube Craig–Post apparatus. After 300 transfers, 6-demethyl-6-deoxytetracycline (IIa) was found in tubes 40–140, while a mixture of "Blue X" and II was formed in tubes 0–39. Several additional runs were made using essentially the same conditions. The contents of tubes 40–140 in each run were combined as a composite fraction. Crystallization of a portion (8.6 g.) of this from methanol (300 ml.) containing concentrated hydrochloric acid (20 ml.) gave chromatographically pure 6-demethyl-6-deoxytetacycline. Material obtained in this way showed λ_{max} (0.01 *N* HCl) 268, 345 mμ; log ε 4.28, 4.18; λ_{max} (0.01 *N* NaOH) 250, 262, 381 mμ; log ε 4.15, 4.19, 4.24. When placed in a preheated bath at 215° the hydrochloride darkened rapidly and decomposed at 220.0–220.2°. When placed in the bath at 210° it decomposed at 217.2–217.6°.

Anal. Calcd. for $C_{21}H_{23}N_2O_7Cl$: C, 55.94; H, 5.14; N, 6.21; Cl, 7.86. Found: C, 55.93; H, 5.24; N, 6.03; Cl, 7.54.

Isolation of "Blue X" By-product (IX).—The crude materials (22 g.) from tubes 0–39 of a number of runs as described above were composited and redistributed as above using 42 tubes of the apparatus for the initial charge and performing 1200 transfers. At the end of this run paper chromatography showed that "Blue X" was present in tubes 100–159; compound IIa was present beyond tube 160, as well as in the withdrawn fractions; two other entities were noted but not characterized (these were probably C-4 epi or 5a,6-anhydro-type compounds). The materials from each tube were isolated by extraction of the upper phase with chloroform, combining of extracts and lower phase, then removal of the organic solvent *in vacuo*. To obtain pure "Blue X" the composite of tubes 100–159 in the above run (465 mg.) was triturated with 2-methoxyethanol ("Methyl Cellosolve") until colorless. The resulting base (320 mg.) was dried *in vacuo* at room temperature overnight. Material obtained in this way was apparently solvated with Methyl Cellosolve.

Anal. Calcd. for $C_{21}H_{26}N_2O_7 \cdot {}^1/_2C_3H_8O_2$: C, 59.18; H, 6.63; N, 6.14. Found: C, 59.55; H, 6.20; N, 5.97.

A hydrochloride was prepared by dissolving 150 mg. of the above base in a mixture of 40 ml. of methanol, 5 ml. of isopropyl alcohol, and 0.05 ml. of concd. HCl. After filtration, an additional 7.5 ml. of concd. HCl was added and the mixture was slowly evaporated in a nitrogen jet to a volume of 13 ml. The white crystalline precipitate was filtered, washed with 1 *N* HCl, then with acetone, and dried at 100° for 3 hr. *in vacuo;* m.p. 222–222.5° dec.

Anal. Calcd. for $C_{21}H_{27}N_2O_7Cl$: C, 55.44; H, 5.98; N, 6.16; Cl, 7.79. Found: C, 55.61; H, 6.01; N, 6.01; Cl, 7.72.

Titration in aqueous ethanol (1EtOH:$2H_2O$) revealed an apparent pK_a' of 3.8, neut. equiv. 461 (calcd. mol. wt. 456). The ultraviolet absorption characteristics of this material differed dramatically from the normal tetracyclines or known derivatives: λ_{max} (MeOH–0.01 *N* HCl) 265, 351 mμ; log ε 4.19, 4.40; λ_{max} (MeOH–0.01 *N* NaOH) 245, 285 (s), 400 mμ; log ε 4.13, 4.06, 4.39; λ_{max} (MeOH–0.01 *N* $NiCl_2$) 280, 358 mμ; log ε 4.14, 4.37; n.m.r. studies on IX (trifluoroacetic acid solution) showed no normal aromatic hydrogen absorption.

A more convenient preparation whereby IX may be obtained in reasonable yield consists in hydrogenation of 7-chloro-6-demethyltetracycline with rhodium catalyst by the following procedure[14]:

7-Chloro-6-demethyltetracycline hydrochloride (20 g.) in methanol (500 ml.) containing 20 ml. of water was hydrogenated with 6 g. of 5% rhodium-on-charcoal catalyst at a temperature of 40° and at 1000 p.s.i. in a stirred autoclave for 6.5 hr. The reaction slurry was filtered free of catalyst and the catalyst washed with methanol. To the resulting solution (745 ml.) there was added 4.8 ml. of concentrated sulfuric acid. Stirring and cooling of the solution resulted in crystallization of 1 g. of product. Upon concentration to a small volume (approximately 100 ml.) an additional 4 g. of crystalline material was obtained. The combined product (5 g.) was noted on paper chromatographic examination to contain a multiplicity of blue fluorescent components. However, the main component was "Blue X." Craig countercurrent distribution (see above) of material obtained in this way resulted in analytically pure "Blue X" identical in every respect with the by-product isolated from 6-demethyl-6-deoxytetracycline.

β-6-Deoxy-5-hydroxytetracycline (IIc).—5-Hydroxytetracycline hydrochloride (70 g.) was dissolved in 800 ml. of methanol and hydrogenated in a stirred autoclave at 50° and 1500 p.s.i. over 25 g. of 5% palladium-on-carbon catalyst for 5 hr. The catalyst was filtered and the filtrate evaporated to dryness to obtain 62 g. of a light-colored solid which assayed approximately 20% β-6-deoxy-5-hydroxytetracycline. (This assay was conducted by measuring the *K. pneumonia* bioassay value before and after treatment of an aliquot sample for 30 min. with boiling methanol containing 20% by volume of concentrated hydrochloric acid. Control experiments indicated that over 99% of any 5-hydroxytetracycline present is destroyed under these conditions while the β-6-deoxy-5-hydroxytetracycline is relatively unaffected.)

The bulk of the crude β-6-deoxy-5-hydroxytetracycline hydrochloride (60 g.) was mixed with 600 ml. of water and the solution adjusted to pH 4.0.[32] The slurry of crude amphoteric material so formed was stirred at 10° for 2 hr. and filtered. The dried cake was extracted first with boiling toluene and then with boiling chloroform to remove impurities soluble in these solvents. An aliquot of the residue (1 g.) was then crystallized as the hydrochloride salt by treating it with 6 ml. of acetone containing 1 ml. of concd. HCl. An analytical sample of the hydrochloride was prepared by recrystallization first from aqueous hydrochloric

(28) A. R. English, private communication.

(29) We are indebted to Dr. T. Lees, Mr. M. Lynch, and associates for the paper chromatographic studies herein.

(30) G. B. Selzer and W. W. Wright, *Antibiotics and Chemotherapy*, **7**, 292 (1957).

(31) For more details on this procedure *cf.* C. R. Stephens, U. S. Patent 2,999,111, September 5, 1961.

(32) It was found later that a more effective separation of β-6-deoxy-5-hydroxytetracycline could be achieved by one extraction of this slurry with an equal volume of ethyl acetate, treatment of the aqueous solution with one equivalent (assay basis) of calcium chloride, then adjusting the pH to 8.2. Under these conditions an essentially quantitative recovery of β-6-deoxy-5-hydroxytetracycline calcium complex is obtained. This may be crystallized as the hydrochloride by treatment with concd. HCl in various solvents—acetone, Ethyl Cellosolve, etc.

acid and then from Ethyl Cellosolve containing hydrochloric acid; m.p. 250–251° dec.; λ_{max} (0.01 *N* MeOH–HCl) 267, 344 mμ; log ε 4.32, 4.17; λ_{max} (0.01 *N* NaOH in MeOH) 250, 380 mμ; log ε 4.22, 4.25; apparent pK_a's 3.4, 7.7, and *ca.* 9.7 (H_2O).

Anal. Calcd. for $C_{22}H_{25}N_2O_8Cl$: C, 55.00; H, 5.22; N, 5.85. Found: C, 54.86; H, 5.35; N, 5.75.

This material may be clearly differentiated from the α-6-deoxy-5-hydroxytetracycline by paper chromatography in a number of systems.

β-6-Deoxytetracycline. A. By Hydrogenolysis of Tetracycline.—The hydrogenation procedure used for β-6-deoxy-5-hydroxytetracycline was applied to tetracycline on a 50-g. scale. The resulting methanolic solution, after removal of the catalyst, was evaporated to dryness. The crude amorphous hydrochloride (42 g.) was dissolved in warm water (800 ml.) and adjusted to pH 4.0. The slurry of crude amphoteric material so formed was filtered and the "gummy" solid repulped in dry ether, filtered, and dried. The crude cake so formed (30 g.) was stirred with methanol (210 ml.) for 1.25 hr. The methanol-insoluble fraction, 15 g., consisting principally of by-product VIII, was composited with other runs for further purification (below). The methanol solution was evaporated *in vacuo* and the crude residue triturated with ether to give 12 g. of solid. This solid was subjected to a Craig countercurrent distribution using essentially the same procedure as that described for 6-demethyl-6-deoxytetracycline (above). β-6-Deoxytetracycline was obtained in the 130 to 190 tube region of a 600-tube Craig run. An aliquot from these tubes (300 mg.) was crystallized from acetone (6 ml.) containing concd. HCl (1 ml.). The analytical sample of β-6-deoxytetracycline hydrochloride decomposed at 245–246° and showed λ_{max} (0.01 *N* HCl in MeOH) 268, 348 mμ; log ε 4.29, 4.18, λ_{max} (0.01 *N* NaOH in MeOH) 249, 380 mμ; log ε 4.23, 4.26.

Anal. Calcd. for $C_{22}H_{25}N_2O_7Cl$: C, 56.95; H, 5.38; N, 6.05. Found: C, 56.64; H, 5.50; N, 6.02.

B. By Hydrogenation of 6-Deoxy-6-demethyl-6-methylenetetracycline.—6-Deoxy-6-demethyl-6-methylenetetracycline[7] hydrochloride (50 g.) was hydrogenated in 67% aqueous dioxane (2.25 l.) at 25° and 1500 p.s.i. over prehydrogenated 5% rhodium-on-charcoal catalyst (50 g.) for 1 hr. The reaction mixture was filtered and concentrated to 235 ml. *in vacuo*. The solution was then adjusted to an apparent pH of 2.0 and subjected to an 11-transfer countercurrent distribution in large separatory funnels between butanol and 0.01 *N* hydrochloric acid, using 250 ml. of butanol and 200 ml. of aqueous phase for each tube—moving the lower phase. After completion of the distribution, 750 ml. of hexane was added to each separatory funnel and the organic solute thus driven into the aqueous phase. The aqueous phases were then freeze-dried and the residues subjected to chromatographic analysis. β-6-Deoxytetracycline was found predominantly in the first four tubes, anhydrotetracycline predominantly in tubes 4–7, and other more highly hydrogenated degradation products in the final tubes. Small amounts of α-6-deoxytetracycline were also noted. The assays showed that an approximately 30% yield of β-6-deoxytetracycline was obtained and an essentially equivalent amount of anhydrotetracycline. Recycle of the concentrates from the various Craig fractions followed by crystallization from methanol–hydrochloric acid resulted in 8.3 g. of pure β-6-deoxytetracycline hydrochloride (identical with material from process A) and 7.4 g. of anhydrotetracycline hydrochloride as well as a considerable amount of mixtures of the two.

Isolation of By-product VIII (4-Dimethylamino-1,4,4a,5,7,8,-9,10,12,12a-decahydro-3,11,12a-trihydroxy-6-methyl-1,10-dioxo-2-naphthacenecarboxamide). A. From Hydrogenolysis Products of Tetracycline.—A composite sample (50 g.) of the methanol-insoluble amphoteric fraction obtained in the isolation of β-6-deoxytetracycline (procedure A above) was slurried in chloroform (300 ml.) and heated under reflux for 30 min. A dark insoluble fraction was filtered off and washed well with warm chloroform. This weighed 15 g. Evaporation of the chloroform gave a crystalline residue which was treated with methanol (100 ml.) and concd. HCl to form the hydrochloride of VIII (27.5 g.). When recrystallized two times from methanol containing a little 5% aqueous HCl (Darco treatment the first time) this material decomposed at 239–240°. The analytical sample showed λ_{max} (0.01 *N* HCl–MeOH) 265, 346 mμ; log ε 4.55, 3.72; λ_{max} (0.01 *N* NaOH–MeOH) 272, 349 mμ; log ε 4.32, 4.54; apparent pK_a's (water) 4.1, 8.4, and *ca.* 12.2.

Anal. Calcd. for $C_{22}H_{27}N_2O_9Cl$: C, 58.6; H, 6.0; N, 6.2; Cl, 7.9. Found: C, 58.6; H, 6.0; N, 5.8; Cl, 8.0.

The base was obtained by dissolving the hydrochloride (1 g.) in 5 ml. of hot water and neutralizing to pH approximately 7.0. The resulting crystals (0.8 g.) were filtered and recrystallized from methanol. The amphoteric compound melted at 209–211° dec; n.m.r. ($CDCl_3$) showed no aromatic hydrogen.

Anal. Calcd. for $C_{22}H_{26}N_2O_8$: C, 64.0; H, 6.3; N, 6.8. Found: C, 63.8; H, 6.4; N, 6.5.

This substance did not undergo any change in ultraviolet absorption when warmed in aqueous alkali (1 *N*). However, when VIII was allowed to stand in aqueous alkaline solution with aldehydes (benzaldehyde, *p*-nitrobenzaldehyde, 5-nitrofufural) a quite characteristic change in ultraviolet absorption was noted consistent with the formation of benzal-type derivatives. The benzal derivatives could be isolated by neutralization of the solution.[33]

B. By Hydrogenation of Anhydrotetracycline.—Anhydrotetracycline hydrochloride (25 g.) in 500 ml. of methanol containing 10 g. of 5% palladium-on-carbon catalyst was stirred with hydrogen at 50° and 1500 p.s.i. pressure in a stainless steel autoclave for 5 hr. Approximately 3 moles of hydrogen was absorbed. The reaction mixture was filtered to remove catalyst and evaporated to dryness. The crude hydrochloride (20 g.) was dissolved in 100 ml. of water and the resulting solution was adjusted to pH 5–6 with aqueous sodium hydroxide. The resulting rather gummy slurry was treated with an equal volume of ether and stirred. This resulted in crystallization of the product. The two-phase system was filtered to obtain compound VIII as a white crystalline solid. Additional product was obtained by evaporation of the ether and by further ether extraction of the aqueous solution. The total yield was 14.0 g. of material identical in infrared absorption spectrum and other physical constants with that obtained directly from tetracycline.

α-6-Deoxy-5-hydroxytetracycline (IIe). A. By Hydrogenation of 6-Demethyl-6-deoxy-6-methylene-5-hydroxytetracycline (IIIa).—6-Demethyl-6-deoxy-6-methylene-5-hydroxytetracycline[7] hydrochloride (50 g.) was suspended in ethanol (1 l.) containing concd. HCl (10 ml.) and stirred in an autoclave for 3.5 hr. with hydrogen (1000 p.s.i. pressure) in the presence of a platinum catalyst (5 g. of PtO_2) at 28°. The catalyst was removed by filtration through a super Cel pad under a nitrogen atmosphere and the filtration apparatus and catalyst cake were washed thoroughly with 300 ml. of ethanol. Evaporation and paper strip assay on an aliquot of this solution revealed an approximate 70% yield of a 1:1 mixture of α- and β-6-deoxy-5-hydroxytetracyclines.

To isolate the α-epimer and combined filtrate (above) was concentrated *in vacuo* to 350 ml., then treated with a solution of 50 g. of 5-sulfosalicylic acid in 125 ml. of methanol. After a 12-hr. crystallization period at 25° there was obtained 10.6 g. of somewhat impure α-6-deoxy-5-hydroxytetracycline sulfosalicylate (contaminents: β-epimer, starting material, and hydrogenation by-products). Evaporation of the mother liquor to 300 ml., then dilution with 125 ml. of water, gave a crude crystalline mixture (18 g.) which on recrystallization from methanol (600 ml. evaporated to 125 ml., 16-hr. crystallization at 25°) furnished an additional 6.5 g. of the α-salt similar in quality to the first crop; total yield of sulfosalicylate: 17.1 g. (25%).

For purification the sulfosalicylate was converted to the base, then to the hydrochloride and other salts. Thus the crude product (17.1 g.) was suspended in 125 ml. of methanol, stirred, and treated with triethylamine until a solution resulted. This was treated rapidly with Darco, filtered, diluted with 50 ml. of water, and adjusted to an apparent pH of 5.7. Crystallization of the base started rapidly. After keeping for 16 hr. at 0° the slurry was filtered and the cake washed with acetone; yield 7.3 g. (63% from crude salt).

An analytical sample of α-6-deoxy-5-hydroxytetracycline hydrochloride was prepared by treating 4.6 g. of the base with 15 ml. of ethanol and 0.8 ml. of concentrated hydrochloric acid, then allowing the solution to crystallize at 0° for 16 hr. There was obtained 4.7 g. of the hydrochloride as a hemihydrate–hemialcoholate. This material chars without melting at about 201°; $\alpha^{25}D$ −110° (*c* 1, 0.01 *N* HCl–methanol); λ_{max} 267, 351 mμ, log ε 4.24, 4.12 (0.01 *N* HCl in methanol).

Anal. Calcd. for $C_{22}H_{24}N_2O_8 \cdot HCl \cdot {}^1/_2C_2H_5OH \cdot {}^1/_2H_2O$: C, 53.85; H, 5.70; N, 5.46; Cl, 6.91; H_2O, 1.76; ethoxyl, 4.39. Found: C, 53.77; H, 5.71; N, 5.49; Cl, 6.64; H_2O, 2.05; ethoxyl, 5.11. When dried to constant weight at 100° under reduced pressure (28 hr.) the sample showed the analysis: Calcd. for $C_{22}H_{24}N_2O_8 \cdot HCl$: C, 54.95; H, 5.24; N, 5.83; Cl, 7.34. Found: C, 55.02; H, 5.30; N, 5.81; Cl, 6.36.

The low chlorine values which are obtained when this material is dried free of solvent indicate that hydrogen chloride is lost from the crystal during the vigorous drying process required to remove solvent.

The base of α-6-deoxy-5-hydroxytetracycline was also converted to the pure sulfosalicylic salt by treatment of 3.6 g. of the material in 50 ml. of methanol with a solution of 3.6 g. of sulfosalicylic acid in 15 ml. of methanol. There was obtained 5.16 g. (96% yield) of the salt. A sample dried at 100° for 28 hr. under reduced pressure showed indefinite decomposition with charring at approximately 201°.

(33) L. H. Conover and C. R. Stephens, U. S. Patent 2,972,630, February 21, 1961.

Anal. Calcd. for $C_{22}H_{24}N_2O_8 \cdot C_7H_6O_6S \cdot {}^1/_2CH_3OH$: C, 52.21; H, 4.75; N, 4.14; S, 4.72; methoxyl, 2.29. Found: C, 52.22; H, 4.80; N, 4.11; S, 4.57; methoxyl, 2.35.

B. By Raney Nickel Desulfurization of 13-Benzylthio-α-6-deoxy-5-hydroxytetracycline (Va).—To 8 g. of Raney nickel suspension was added a solution of 1 g. of 13-benzylthio-α-6-deoxy-5-hydroxytetracycline[10] in 30 ml. of methanol and 4 drops of concd. HCl. The slurry was heated under reflux with stirring for 8 hr., then separated by centrifugation, and the catalyst washed by centrifugation (3 × 20 ml. of methanol). The organic layers were combined, evaporated to dryness, slurried in ether, filtered, and dried to give 890 mg. of crude solid. The product was crystallized by dissolution in 20 ml. of methanol, treatment with 1 g. of sulfosalicylic acid then removal of inorganic salts by filtration. The solution was then left to crystallize at room temperature for 16 hr. There was obtained 500 mg. (43% yield) of reasonably pure α-6-deoxy-5-hydroxytetracycline sulfosalicylate—identical in infrared absorption, chromatographic behavior, etc., with the material from procedure A.

Hydrogenation of 11a-Fluoro-6-methylene-5-hydroxytetracycline (IV).—A slurry of 250 mg. of 5% rhodium-on-carbon in 25 ml. of 0.01 *N* hydrochloric acid was prehydrogenated at 23° and atmospheric pressure. After saturation of the catalyst, 250 mg. of 11a-fluoro-6-methylene-5-hydroxytetracycline[7] hydrofluoride was added, and the hydrogenation continued for 2 days. The hydrogen uptake was very slow during the last 36 hr. and consisted over-all of 19 ml. (75% of theory). The reaction mixture was filtered and freeze-dried to yield 125 mg. of a tan solid with ultraviolet absorption maxima (0.01 *N* HCl in MeOH) at 268 and 344 mμ. Paper chromatographic analysis showed the product to be predominantly β-6-deoxy-5-hydroxytetracycline containing only a minor quantity of α-6-deoxy-5-hydroxytetracycline.

An identical experiment performed on 6-methylene-5-hydroxytetracycline gave an equal mixture of the α- and β-6-deoxy epimers.

α-6-Deoxytetracycline (IId) from 13-Benzylthio-α-6-deoxytetracycline (Vb).—13-Benzylthio-α-6-deoxytetracycline *p*-toluenesulfonate[10] (58 g.) was heated under reflux in 3.33 l. of ethanol containing 106 ml. of concd. HCl in the presence of 580 g. of Raney nickel for 3 hr. A nitrogen atmosphere was maintained. The reaction mixture was cooled to room temperature, filtered, and the filter cake washed with ethanol. The combined filtrates were evaporated to dryness under reduced pressure to yield a yellow-green solid (146 g.). This was distributed between butanol (1 l.) and aqueous hydrochloric acid of final pH 1.5 (1 l.). The aqueous phase was then saturated with sodium chloride and extracted with three 200-ml. portions of butanol. The combined butanol phases were extracted once with water (200 ml.), then concentrated to dryness under reduced pressure to yield a green-yellow solid (37.6 g.). Paper chromatographic analysis of this crude product showed it to contain a mixture of α-6-deoxytetracycline and its C-4 epimer, but no β-6-deoxytetracycline.

In order to effect the conversion[34] of the C-4 epimer to α-6-deoxytetracycline, the crude product was ground with 25 g. of anhydrous calcium chloride and heated under reflux in 290 ml. of butanol and 14 ml. of β-aminoethanol for 3 hr. under nitrogen. The reaction mixture was filtered and evaporated at reduced pressure to yield a gummy orange-yellow cake (65.3 g.). The crude product was stirred at room temperature in 650 ml. of butanol and 82 ml. of 5% aqueous hydrochloric acid for 30 min., then filtered. The filtrate was evaporated to dryness under reduced pressure to yield a yellow residue (28 g.). This material was subjected to a 6-tube, 10-transfer countercurrent distribution between butanol and 0.1 *N* hydrochloric acid (350 ml. each phase/tube) moving the aqueous phase. Each tube was then treated with hexane (700 ml.), equilibrated, and the lower phase separated, and freeze-dried. The residues from tubes 4 and 5 were crystallized from acetone. This material (3.8 g.) still contained inorganic contaminants. For purification, it was dissolved in 50 ml. of hot water, *p*-toluenesulfonic acid (7.2 g.) was added, and the crystalline α-6-deoxytetracycline *p*-toluenesulfonate which separated was collected (0.45 g.): λ_{max} (MeOH–0.01 *N* HCl) 269, 352 mμ; log ε 4.28, 4.20. Paper chromatographic analysis at all stages showed this purified material to be the predominant product of the reaction—the minute recovery being due to the difficulty in removing the large excess of inorganic material.

Anal. Calcd. for $C_{22}H_{24}O_7N_2 \cdot C_7H_7SO_3H$: C, 57.99; H, 5.37; N, 4.66. Found: C, 57.73; H, 5.43; N, 4.62.

4-Dedimethylamino-6-demethyl-6-deoxytetracycline.—6-Demethyl-6-deoxytetracycline (amphoteric, 34.5 g.) was slurried in 2.1 l. of acetone containing 150 ml. of methyl iodide. The reaction mixture was stirred for 72 hr. at room temperature then filtered free of a small amount of insoluble material. The solution was evaporated to dryness *in vacuo* and the residue slurried with ether to provide, on filtration and air-drying, 44.7 g. (96%) of crude 6-demethyl-6-deoxytetracycline methiodide. The crude methiodide (40 g.) was dissolved in 1200 ml. of 50% aqueous acetic acid at room temperature. Zinc dust (24 g.) was added portionwise and the mixture stirred for 15 min.[15] Acetone (1.2 l.) was added and the excess zinc removed by filtration. The filtrate was treated with aqueous hydrochloric acid (5%, 1.2 l.) and the mixture concentrated under a water aspirator to *ca.* 2.4 l. The resulting slurry was transferred to a separatory funnel and the product extracted into methylene chloride (2 l. in three portions). The methylene chloride was washed five times with 250-ml. portions of 2.5% aqueous hydrochloric acid, dried over anhydrous sodium sulfate, filtered, and evaporated to dryness to yield microcrystalline 6-demethyl-6-deoxy-4-dedimethylaminotetracycline (21.4 g., 80%). For analytical sample preparation the material was taken up in ether, filtered, and evaporated to dryness; λ_{max} 221, 264, 303 mμ, log ε 4.25, 4.26, 4.19 (MeOH–0.01 *N* HCl).

Anal. Calcd. for $C_{19}H_{17}NO_7$: C, 61.5; H, 4.6; N, 3.8. Found: C, 61.5; H, 4.9; N, 3.7.

11a-Fluoro-6-demethyl-6-deoxy-4-dedimethylaminotetracycline.—6-Demethyl-6-deoxy-4-dedimethylaminotetracycline (9.25 g.) was slurried in 1.25 l. of methanol and cooled to *ca.* 5° in an ice bath. Sodium methoxide (2.70 g.) dissolved in 50 ml. of methanol was added. Complete solution occurred. Perchloryl fluoride was bubbled slowly through the solution with gentle stirring until the apparent pH was 6.0. Ice-water (1.25 l.) was added (noticeable gas evolution). The product crystallized while standing 2 hr. in the ice bath and was recovered by filtration; yield, including a second crop, 8.0 g. (82%). A sample was recrystallized from hot methanol for analysis; λ_{max} (CH_3OH–0.01 *N* HCl) 265, 348 mμ; log ε 4.40, 3.57.

Anal. Calcd. for $C_{19}H_{16}FNO_7$: C, 58.6; H, 4.1; N, 3.6; F, 4.9. Found: C, 58.2; H, 4.3; N, 3.4; F, 5.1.

11a-Fluoro-6-demethyl-6-deoxytetracyline.—A solution of 2.3 g. (0.005 mole) of 6-demethyl-6-deoxytetracycline hydrochloride in 100 ml. of water containing 15 ml. (0.005 mole) of 1 *N* sodium hydroxide was placed in a 250-ml. 3-neck flask equipped with a magnetic stirrer, gas inlet and outlet tubes (including bubble counters), and a pH meter probe. Perchloryl fluoride was passed into the solution at room temperature. After 55 min. the initial pH (11) had changed to 8.0 and precipitation had set in. After 260 min. the pH had reached 5.6. At this time the addition of perchloryl fluoride was interrupted. The excess reagent was swept out of the flask with a stream of nitrogen, the flask was chilled in an ice bath, and the precipitate filtered and thoroughly washed with water, then with ether. The product was dried *in vacuo* over phosphorus pentoxide; yield 15.2 g. (71%). Material obtained in this way showed λ_{max} 270, 350 mμ; log ε 4.45, 3.71 (MeOH–0.01 *N* HCl).

Anal. Calcd. for $C_{21}H_{21}N_2O_7F \cdot 0.5H_2O$: C, 57.2; H, 4.04; N, 6.35; F, 4.31. Found: C, 57.1; H, 4.91; N, 6.17; F, 4.03.

11a-Fluoro-β-6-deoxy-5-hydroxytetracycline Hydrochloride.—A solution of 4.44 g. (0.925 mmole) of β-6-deoxy-5-hydroxytetracycline hydrochloride in 100 ml. of water was placed in a 250-ml. 3-necked flask equipped with a stirrer, gas inlet and outlet tubes (including bubble counters), and a pH meter probe. The solution was cooled in an ice bath, flushed with nitrogen, and treated with 10 ml. (2 millimoles, 2.16 equiv.) of 2 *N* sodium hydroxide. The pH of the resulting solution was 9.2. Perchloryl fluoride was passed into the solution until the pH reached 7.2 (requiring 95 min.). Excess perchloryl fluoride was then swept out with a stream of nitrogen. The solution was adjusted to pH 2.5 with 2 *N* hydrochloric acid and extracted three times with 100-ml. portions of butanol. Evaporation of the solution to a small volume yielded 2.5 g. (53%) of pure crystalline product, λ_{max} (MeOH–0.01 *N* HCl) 269, 345 mμ; log ε 4.12, 3.59.

Anal. Calcd. for $C_{22}H_{23}N_2O_8F \cdot HCL \cdot 0.5H_2O$: C, 51.9; H, 4.97; N, 5.54. Found: C, 52.0; H, 5.22; N, 5.36.

11a-Chloro-6-demethyl-6-deoxytetracycline (XI).—A solution of 6-demethyl-6-deoxytetracycline hydrochloride (2.7 g.) in 80 ml. of H_2O was treated with 840 mg. of N-chlorosuccinimide. The solution was stirred at room temperature for 15 min., treated with Darco G-60, and filtered. The product was precipitated as the sulfate salt by the addition of 10 ml. of concd. H_2SO_4. Cooling, filtration, and washing, first with acetone, then with ether, yielded 2.2 g. of white crystalline 11a-chloro-6-demethyl-6-deoxytetracycline sulfate. For analysis the sulfate was converted to the nitrate salt by stirring in 25 ml. of 5% HNO_3 for 24 hr. The ultraviolet spectrum showed λ_{max} (MeOH–0.01 *N* HCl) 270, 350 mμ; log ε 4.43, 3.61. The infrared spectrum (KBr pellet) showed a strong 5.70 μ carbonyl band.

Anal. Calcd. for $C_{21}H_{21}N_2O_7Cl \cdot HNO_3$: C, 49.27; H, 4.33; N, 8.21; Cl, 6.98. Found: C, 49.42; H, 4.30; N, 7.97; Cl, 6.77.

7- and 9-Chloro-6-demethyl-6-deoxytetracycline.—A solution of 20 g. of 6-demethyl-6-deoxytetracycline hydrochloride in 150 ml. of trifluoroacetic acid was treated with 12 g. of N-chlorosuccinimide, stirred at 60° for 2 hr., then poured into 2.5 l. of cold ether with stirring. Filtration gave 23.1 g. of a crude mix-

(34) M. Noseworthy, U. S. Patent 3,009,956, November 21, 1961.

ture of 7- and 9-chlorinated 11a-chloro-6-demethyl-6-deoxytetracycline salts, λ_{max} (KBr) 5.73 μ; λ_{max} (MeOH–0.01 *N* HCl) 223, 270, 358 mμ.

Twenty grams of the above mixture in 200 ml. of methanol was hydrogenated briefly in the presence of 500 mg. of 5% palladium-on-carbon at one atmosphere. Removal of the catalyst, evaporation of the solvent, and crystallization from methanolic sulfuric acid gave 9.2 g. of crude 7- and 9-chloro-6-demethyl-6-deoxytetracycline sulfates contaminated with starting material. A portion of the crystalline mixture (5.5 g.) was purified by an 800-tube countercurrent distribution using a two-phase system prepared by equilibrating equal volume ratios of chloroform, carbon tetrachloride, methanol, and water (10 ml. of each phase per tube). The material was loaded in the first 16 tubes by dissolving in upper phase solvent and adjusting the pH to 6.0. The distribution was followed by paper chromatography of representative tubes. Tubes 90–139 were combined and evaporated to dryness to yield 3.54 g. of 7-chloro-6-demethyl-6-deoxytetracycline. The material was crystallized from methanol containing excess *p*-toluenesulfonic acid and the resulting *p*-toluenesulfonate salt recrystallized from methanol to provide an analytical sample. The ultraviolet absorption spectrum in acidic methanol showed λ_{max} 223, 268, 345, 365 mμ; log ϵ 4.51, 4.37, 4.17, 4.17.

Anal. Calcd. for $C_{21}H_{21}N_2O_7Cl \cdot C_7H_8O_3S$: C, 54.15; H, 4.71; N, 4.51; Cl, 5.71; S, 5.16. Found: C, 53.98; H, 4.77; N, 4.40; Cl, 5.86; S, 5.29.

Withdrawn tubes 600–699 were combined and evaporated to dryness to give 0.2 g. of the 9-chloro isomer XIVa which was also crystallized from methanol as the *p*-toluenesulfonic acid salt. Recrystallization from methanol gave an analytical sample as a hemimethanolate, m.p. 268–270° dec.; λ_{max} (MeOH–0.01 *N* HCl) 269, 349 mμ; log ϵ 4.53, 4.30.

Anal. Calcd. for $C_{21}H_{21}N_2O_7Cl \cdot C_7H_8O_3S \cdot 0.5CH_3OH$: C, 53.72; H, 4.90; N, 4.40; Cl, 5.57; S, 5.03; OCH_3, 1.18. Found: C, 53.60; H, 4.89; N, 4.37; Cl, 5.41; S, 4.92; OCH_3, 0.80.

Withdrawn tubes 400–599 yielded 0.9 g. of 6-demethyl-6-deoxytetracycline (IIa).

Methylation–Oxidation of 7-Chloro-6-demethyl-6-deoxytetracycline.—A suspension of 0.5 g. of pure 7-chloro-6-demethyl-6-deoxytetracycline sulfate in 10 ml. of water was rendered strongly basic with 50% sodium hydroxide solution. Dimethyl sulfate (5 ml.) was added with stirring over a 2-hr. period, keeping the pH of the solution above 10.0 by adding additional caustic as required. The reaction was heated on a steam bath for 1 hr. and then treated with a large excess of saturated aqueous potassium permanganate solution over a 3-hr. period. The mixture was heated on a steam bath with stirring overnight, cooled to room temperature, and treated with sufficient sodium sulfite to dissolve the manganese dioxide. The solution was continuously extracted with ether overnight and the ether extract evaporated *in vacuo* to a dark-colored gum. This was sublimed at 140–160° (0.13 mm. pressure). The sublimate was resublimed at 140° (0.13 mm.) to yield 10 mg. of 3-chloro-6-methoxyphthalic anhydride (XII), m.p. 187°, identical with an authentic sample prepared by a similar degradation[23] on 7-chlorotetracycline.

11a-Bromo-6-demethyl-6-deoxytetracycline.—A solution of 450 mg. of 6-demethyl-6-deoxytetrracycline (IIa) in 25 ml. of water was treated with 185 mg. of N-bromoacetamide, stirred for 30 min., then treated with 2 ml. of concd. H_2SO_4, and allowed to crystallize overnight. The material was collected, washed with acetone, and dried to provide 440 mg. of crude, crystalline 11a-bromo-6-demethyl-6-deoxytetracycline sulfate, λ_{max} (KBr pellet) 5.72 μ; λ_{max} (MeOH–0.01 *N* HCl) 270, 350 mμ; log ϵ 4.44, 3.65. The compound proved to be too unstable for recrystallization. Starting material could be readily regenerated in a variety of solvents (acetone, etc.) since this material is an active brominating agent.

7,11a-Dibromo-6-demethyl-6-deoxytetracycline.—A solution of 0.5 g. of 6-demethyl-6-deoxytetracycline (IIa) in 5 ml. of trifluoroacetic acid was stirred and treated dropwise with a solution of 0.4 g. of bromine in 2 ml. of acetic acid. The resulting heavy suspension was stirred for 30 min., filtered, washed with ether, and dried to yield 0.8 g. of crude 7,11a-dibromo-6-demethyl-6-deoxytetracycline hydrobromide, λ_{max} (MeOH–0.01 *N* HCl) 272, 364 mμ; λ_{max} (KBr pellet) 5.72 μ. This compound proved to be quite unstable,[35] but could be recrystallized from cold tetrahydrofuran and chloroform.

Anal. Calcd. for $C_{21}H_{20}N_2O_7Br_2 \cdot HBr$: C, 38.61; H, 3.24; N, 4.29; Br, 36.71. Found: C, 38.03; H, 3.61; N, 4.15; Br, 38.03.

7-Bromo-6-demethyl-6-deoxytetracycline.—A solution of 6-demethyl-6-deoxytetracycline (2.0 g.) in 15 ml. of trifluoroacetic acid was treated with 1.28 g. of bromine in 8 ml. of acetic acid. The suspension was stirred on a steam bath for 10 min. and the hot solution poured into 200 ml. of ether. Filtration, washing, and drying yielded 2.9 g. of amorphous solid that was crystallized by dissolving in 10 ml. of methanol, 10 ml. of acetone, and 1 ml. of 48% HBr and heating the solution on a steam bath. After cooling there was obtained 1.8 g. of 7-bromo-6-demethyl-6-deoxytetracycline hydrobromide, λ_{max} (MeOH–0.01 *N* HCl) 268, 345, 365 mμ; log ϵ 4.30, 4.12, 4.12; λ_{max} (MeOH–0.01 *N* NaOH) 243, 382 mμ; log ϵ 4.23, 4.10. Recrystallization from dimethylformamide–water and then from ethanol yielded the analytical sample.

Anal. Calcd. for $C_{21}H_{21}N_2O_7Br \cdot HBr$: C, 43.92; H, 3.86; N, 4.88; Br, 27.84. Found: C, 43.96; H, 3.99; N, 4.82; Br, 28.10.

The 7-bromo structure was assigned this compound on the basis of its characteristic (split terminal peak) ultraviolet absorption (similar to that of the 7-chloro compound) and from the observation that nitration gave a product with a characteristic 9-nitro chromophore (λ_{max} 239, 285, 446 mμ in 0.01 *N* NaOH in MeOH). No concerted effort was made to isolate a 9-bromo component from the crude reaction product.

9-Amino-6-demethyl-6-deoxytetracycline.—9-Nitro-6-demethyl-6-deoxytetracycline[24] (20 g.) was dissolved in methanol (500 ml.) containing 10 ml. of concentrated hydrochloric acid; 500 mg. of platinum oxide was added and the resulting mixture was hydrogenated at room temperature and atmospheric pressure in a glass apparatus equipped with a magnetic stirrer until the theoretical amount of hydrogen was absorbed. The catalyst was removed by filtration and the filtrate was concentrated *in vacuo* to about 200 ml. The pH of the resulting solution (measured on a 1:1 aqueous dilution) was adjusted to an apparent value of 3.3 with triethylamine. The crystal mass which resulted was left for several hours at 5°, filtered, and washed with cold (0–5°) methanol. For analysis the crystals (9.4 g.) were dried *in vacuo* at room temperature; λ_{max} (MeOH–0.01 *N* HCl) 266, 353 mμ; log ϵ 4.43, 3.90.

Anal. Calcd. for $C_{21}H_{23}N_3O_7 \cdot HCl \cdot H_2O$: C, 52.08; H, 5.62; N, 8.67. Found: C, 51.96; H, 5.32; N, 8.67.

The 9-amino hydrochloride was converted to the base by suspending 3 g. of the salt in 20 ml. of water and adjusting the pH (while the solution was under nitrogen) to 8.8. The solution so formed was filtered under nitrogen and the pH then adjusted to 6.0 with dilute hydrochloric acid. The crystalline suspension which resulted was stirred in an ice bath (still under nitrogen) for several hours. The crystals were filtered, thoroughly washed with cold water, and dried *in vacuo* over phosphorus pentoxide. The yield was 2.51 g. (90%).

Anal. Calcd. for $C_{21}H_{23}N_3O_7 \cdot H_2O$: C, 56.37; H, 5.64; N, 9.4. Found: C, 56.07; H, 5.75; N, 9.87.

7-Amino-6-demethyl-6-deoxytetracycline[1b,2c] was prepared in a very similar manner from the 7-nitro analog.[24]

9-Iodo-6-demethyl-6-deoxytetracycline.—9-Amino-6-demethyl-6-deoxytetracycline hydrochloride (1.4 g.) was dissolved in 2 *N* H_2SO_4 (10 ml.) and the solution cooled in an ice bath and treated slowly with 3.5 ml. of 1 *N* sodium nitrite solution, introduced below the surface of the liquid. After a stirring period of 15 min., 30 mg. of urea was added to destroy excess nitrous acid. The solution was then treated with 1 ml. of 47% hydriodic acid. A yellow solid precipitated. The mixture so formed was stirred at room temperature for 30 min., then warmed gradually to 50° and stirred at this temperature for 20–30 min. Nitrogen evolution was noted as well as a darkening in color. The slurry was then cooled and the pH adjusted to 5.5. After stirring at ice bath temperature for several hours the slurry was filtered and the precipitate washed with water and dried *in vacuo* over P_2O_5. The yield of crude material was 1.44 g.

The crude product was suspended in water and treated with 2 *N* hydrochloride until solution was achieved. Sodium bisulfite (a small amount) was then added and the pH readjusted to 5.5. This caused a distinct lightening in color. The suspension so formed was extracted four times with excess ethyl acetate. The ethyl acetate extract was dried over sodium sulfate and concentrated *in vacuo* until crystallization started. The slurry was then heated until solution occurred, a small amount of methanol added, and the resulting solution left overnight in the refrigerator. The crystals were filtered and washed with ether. There was obtained 500 mg. of fine yellow needles, λ_{max} (MeOH–0.01 *N* HCl) 236, 266, 350 mμ; log ϵ 4.44, 4.40, 4.21.

Anal. Calcd. for $C_{21}H_{21}N_2O_7I \cdot CH_3OH$: C, 46.2; H, 4.4; N, 4.9; I, 22.2. Found: C, 46.7; H, 4.3; N, 4.8; I, 21.3.

9-Chloro-6-demethyl-6-deoxytetracycline (XIVa) by the Sandmeyer Reaction.—9-Amino-6-demethyl-6-deoxytetracycline hydrochloride (1.04 g.) was dissolved in a mixture of 5 ml. of water and 2 ml. of concentrated hydrochloric acid. The solution was cooled to 0°, stirred, and treated gradually with a solution of 150 mg. of sodium nitrite in 2 ml. of water, introduced below the surface with a pipet. After 20 min., 50 mg. of urea was added to

(35) Dissolution in acetone containing some 48% HBr resulted in 7-bromo-6-demethyl-6-deoxytetracycline and bromoacetone. Heating the compound in tetrahydrofuran gave a 5a,6-anhydro compound presumably 7-bromo-6-demethylanhydrotetracycline.

destroy excess nitrite. A solution of freshly prepared cuprous chloride (from 600 mg. of cupric sulfate) in 1 ml. of concentrated hydrochloric acid was added. The mixture was stirred for 5 min. at room temperature, then warmed gradually to 50° in a water bath and stirred at this temperature for 50 min. The solution so formed was cooled and extracted with butanol. Evaporation of the solvent gave 845 mg. of crude 9-chloro compound. This was purified by dissolving in aqueous hydrochloric acid and treatment with *p*-toluenesulfonic acid to form a *p*-toluenesulfonate salt which was then recrystallized from aqueous ethanol. The 9-chloro compound obtained in this way was identical in every respect with the minor product obtained in the N-chlorosuccinimide chlorination of 6-deoxy-6-demethyltetracycline (see above).

A 9-bromo analog (XIVb) was prepared in an identical manner to that described above substituting hydrobromic for hydrochloric acid. 7-Halo compounds (bromo and chloro) were also prepared from 7-amines by this procedure.

O^{10}-Benzenesulfonyl-6-demethyl-6-deoxytetracyclinonitrile (XV).—A solution of 45.0 g. of 6-demethyl-6-deoxytetracycline hydrochloride in 200 ml. of reagent pyridine was cooled to 6° in an ice bath and treated dropwise with stirring with 45.0 ml. of benzenesulfonyl chloride. A strongly exothermic reaction was noted. The temperature rose to 20°. The addition of benzenesulfonyl chloride was maintained at a rate that kept the temperature at 17° or below. After this addition the reaction solution stood for 1.5 hr. at room temperature and was then diluted with 400 ml. of water when a precipitate formed. The diluted mixture was vigorously stirred for 0.5 hr., then filtered and the solid washed with water. There was obtained 42.4 g. of a microcrystalline yellow solid. This was recrystallized by dissolving in 200 ml. of hot dimethylformamide, treating with Darco G60, filtering, and washing the carbon thoroughly with hot acetone. The filtrate was then treated with excess acetone and stirred at room temperature until crystallization occurred. The mixture was cooled on an ice bath. The slurry was filtered and washed with a small amount of acetone. There was obtained 18.1 g. of pale yellow crystals. For analysis a 0.5-g. sample of this material was dissolved in 10 ml. of dimethylformamide and filtered. The filtrate was treated with 100 ml. of methanol, heated to the boiling point on a steam bath, then left in the refrigerator for 16 hr. to crystallize. The yellow crystals were filtered, washed with methanol then acetone, and dried at 100° *in vacuo* over P_2O_5 for 16 hr.; m.p. 236–239° dec., weight 0.38 g.; λ_{max} (MeOH–0.01 *N* HCl) 281, 348 mμ; log ε 4.15, 4.07.

Anal. Calcd. for $C_{27}H_{24}N_2O_8S \cdot 0.5H_2O$: C, 59.43; H, 4.62; N, 5.14; S, 5.88. Found: C, 59.39; H, 4.73; N, 5.33; S, 6.05.

6-Demethyl-6-deoxytetracyclinonitrile (XVII).—A solution of 1.9 g. of O^{10}-benzenesulfonyl-6-demethyl-6-deoxytetracyclinonitrile in 30.4 ml. of liquid HF was left at room temperature overnight in a screw-cap polyethylene bottle. The liquid HF solution was evaporated in a nitrogen stream to a small volume (2–5 ml.) and this oil was diluted with excess anhydrous ether. The greenish yellow solid so obtained was filtered, washed with ether, then acetone, then again with ether. There was obtained 1.6 g. of amorphous material. Paper chromatography on this indicated the formation of a smll amount of 6-demethyl-6-deoxytetracycline as well as the nitrile.

For analysis a 100-mg. sample of crude nitrile was dissolved in 60 ml. of hot methanol and filtered. The solution was evaporated to approximately 2 ml. in a dust-free atmosphere, then left in the refrigerator to crystallize overnight. There was obtained 55 mg. of pale yellow crystals, m.p. 266–267° dec.; λ_{max} (MeOH–0.01 *N* HCl) 277, 353; log ε 4.13, 4.13.

Anal. Calcd. for $C_{21}H_{20}N_2O_8 \cdot 0.5H_2O$: C, 62.21; H, 5.22; N, 6.91. Found: C, 62.32; H, 5.69; N, 6.80.

6-Demethyl-6-deoxytetracyclinonitrile may be reconverted to the parent amide IIa *via* the N^2-*t*-butyl compound (see below) or by direct acid hydrolysis. A preferred procedure involves heating the nitrile (2 g.) in boron trifluoride etherate (25 ml.) containing a little water (1 cc.) at 100° for 12 hr., then pouring the solution into excess ether. Large amounts of the C-4 epimer of IIa are also produced.

O^{10}-Benzenesulfonyl-N^2-*t*-butyl-6-demethyl-6-deoxytetracycline (XVI).—A suspension of 10.0 g. of O^{10}-benzenesulfonyl-6-demethyl-6-deoxytetracyclinonitrile in 100 ml. of glacial acetic acid was treated with 18.0 ml. of concentrated sulfuric acid and stirred at 40–45° for 15 min. in an effort to obtain a clear solution; complete solution did not occur. The mixture was cooled at 10–15° in an ice–water bath and treated with a stream of isobutylene gas. After 9.0 g. of isobutylene had been adsorbed into the slurry the reaction flask was stoppered and allowed to sit overnight in the refrigerator. The next morning a slight amount of solid was still present. This was removed by filtration and the filtrate was extracted four times with 250-ml. portions of hexane to remove hydrocarbon polymers. The acetic acid solution was then diluted with 200 ml. of water. A pale yellow solid separated. This mixture was extracted three times with 100-ml. portions of chloroform. Bad emulsions were noted which required filtration at each extraction. The combined chloroform extracts were dried over sodium sulfate and the chloroform evaporated *in vacuo*. The yellowish green oil which remained was triturated with a small amount of methanol whereupon it crystallized. The crystals were transferred to a funnel with acetone and washed with acetone on the funnel; weight 6.6 g. For analysis, 3.3 g. of this crude product was dissolved in 500 ml. of boiling acetone, filtered, and allowed to sit in the refrigerator overnight. Pale yellow crystals separated. These were filtered and washed with acetone; weight 1.6 g.; λ_{max} (0.01 *N* HCl in methanol) 258, 350 mμ; log ε 4.29, 4.07. This material was a sulfate salt, m.p. 207–210° dec.

Anal. Calcd. for $C_{31}H_{34}O_9N_2S \cdot 0.5H_2SO_4 \cdot 0.5H_2O$: C, 55.68; H, 5.43; N, 4.19; S, 7.19. Found: C, 55.72; H, 5.48, N, 4.17; S, 7.05.

O^{10}-Benzenesulfonyl-N^2-*t*-butyl-6-demethyl-6-deoxytetracycline undergoes facile reconversion to 6-demethyl-6-deoxytetracycline (IIa) by the action of concentrated sulfuric acid[36] (25°, 1 hr.).

N^2-*t*-Butyl-6-demethyl-6-deoxytetracycline (XVIII).—A solution of 1.0 g. of 6-demethyl-6-deoxytetracyclinonitrile in 10 ml. of glacial acetic acid containing 1.8 ml. of concentrated sulfuric acid was cooled to 10° and treated with 0.9 g. of isobutylene gas. The resulting green solution was tightly stoppered and placed in the refrigerator overnight. It was then extracted three times with 25-ml. portions of hexane to remove hydrocarbon polymers. The acetic acid solution was then diluted with 300 ml. of water, when a light yellow precipitate formed. The slurry was extracted four times with 20-ml. portions of chloroform. The chloroform extracts were combined and dried over anhydrous sodium sulfate. The chloroform was evaporated to dryness *in vacuo*, and residual oil was triturated with a small amount of methanol, then scratched to induce crystallization. The mixture was left for 24 hr. to crystallize fully. The crystalline mass was transferred to a filter funnel with anhydrous ether. The cake was washed three times with anhydrous ether and dried *in vacuo;* weight 1.0 g.

For analysis, 0.5 g. of this crude N-*t*-butyl amide was dissolved in 15 ml. of hot methanol. The methanol solution was filtered, then evaporated at the b.p. to a volume of about 7 ml. and left to crystallize. The pale yellow crystalline product filtered and washed with ether, then dried at 50° *in vacuo*, sintered at 168°, m.p. 189–192° dec.; λ_{max} (MeOH–0.01 *N* HCl) 266, 349 mμ; log ε 4.28, 4.19.

Anal. Calcd. for $C_{25}H_{30}N_2O_7 \cdot CH_3OH \cdot 0.5H_2SO_4$: C, 56.61; H, 6.40; N, 5.08; S, 2.91; OCH_3, 5.63. Found: C, 56.44; H, 6.39; N, 5.27; S, 3.42; OCH_3, 5.16.

Analogous to the preparation of amides by Benson and Ritter,[36] *in strong acid solution* (*e.g.*, concd. H_2SO_4 at room temperature for 1 hr.), N^2-*t*-butyl-6-demethyl-6-deoxytetracycline undergoes facile elimination of the *t*-butyl group with the regeneration of 6-demethyl-6-deoxytetracycline (IIa).

Sulfonation of 11a-Chloro-6-demethyl-6-deoxytetracycline.—11a-Chloro-6-demethyl-6-deoxytetracycline (1 g.) was dissolved in 5 ml. of concd. H_2SO_4 at room temperature. The solution was stirred for 4 hr., then added dropwise to 300 ml. of cold ether while stirring in an ice bath. The slurry was left overnight stirring in an ice bath. It was then filtered and reslurried four times with ether, then washed with anhydrous ether to dry. There was obtained 1.3 g. of an amorphous solid.

Anal. Found: N, 4.1; S, 10.0.

This composition corresponds to a crude sulfonated sulfate salt, *i.e.*, 2 equiv. of sulfate for two nitrogens.

For purification 1 g. of this crude material was boiled in 200 ml. of methanol. A dark-colored crystalline insoluble material was filtered (190 mg.) and the filtrate was concentrated on a steam bath to a reduced volume. The concentrated solution was left at room temperature overnight. Nicely crystalline product (410 mg.) separated. This was isolated and washed with methanol. This material showed a neutral equivalent of 530 and apparent pK_a's in 50% dimethylformamide–water of 4.35 and 7.80. The ultraviolet absorption curve showed λ_{max} 229, 270, 348 mμ (methanol–0.01 *N* HCl). These properties are consistent with a D-ring sulfonic acid zwitterion structure which could readily form by solvolysis of the original crude salt.

Anal. Calcd. for $C_{21}H_{21}N_2ClSO_{10} \cdot 0.5H_2O$: C, 46.97; H, 4.13; N, 5.22; Cl, 6.42; S, 5.96. Found: C, 47.08; H, 4.21; N, 5.09; Cl, 6.47; S, 6.23.

A less pure sulfonated material was obtained by the direct action of concd. H_2SO_4 (10 ml.) on 6-demethyl-6-deoxytetracycline (4.7 g.) for 48 hr. at room temperature. The solution so obtained was poured into water (250 ml.) and the insoluble material (0.8 g.) rejected. The water solution was then adjusted to pH 2.5 to give 3.18 g. of crude sulfonate which appeared (paper chromatography) to be identical with product obtained by sodium hydrosulfite dehalogenation of the above 11a-chloro sulfonate.

(36) F. R. Benson and J. J. Ritter, *J. Am. Chem. Soc.*, **71**, 4128 (1949).

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[CONTRIBUTION FROM THE RESEARCH LABORATORIES OF THE UPJOHN CO., KALAMAZOO, MICH.]

The Chemistry of Actinospectacin. IV. The Determination of the Structure of Actinospectacin[1]

BY PAUL F. WILEY, ALEXANDER D. ARGOUDELIS, AND HERMAN HOEKSEMA

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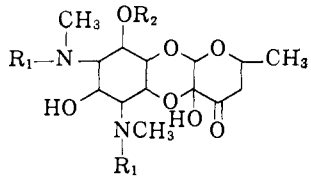
A structure is proposed for the antibiotic actinospectacin, and the work supporting the proposed structure is discussed.

Actinospectacin[2-5] is a broad spectrum antibiotic produced by an actinomycete, *Streptomyces spectabilis*. The structure of this antibiotic aside from stereochemistry has been determined to be that represented by I, and this paper discusses the evidence on which this proposed structure is based.



I, $R_1 = R_2 = H$
II, $R_1 = CH_3CO$, $R_2 = H$
III, $R_1 = R_2 = CH_3CO$
IV, $R_1 = H$, $R_2 = CH_3CO$
V, $R_1 = C_2H_5NHCO$, $R_2 = H$

Crystalline actinospectacin hexahydrate has the molecular formula $C_{14}H_{24}N_2O_7 \cdot 6H_2O$.[6] Both of the nitrogen atoms are basic having pK_a' values of 6.78 and 8.80. This is in agreement with the formation by actinospectacin of a dihydrochloride and a neutral sulfate.[4] Functional group analyses demonstrated the presence of one C-methyl group and two N-methyl groups, but methoxyl groups are absent. The infrared spectrum has several bands in the 3200–3500 cm.$^{-1}$ region attributable to hydroxyl and amino groups, but, in the hydrated state there is no absorption in the carbonyl region. However, the infrared spectra of rigorously dried actinospectacin and its salts have an absorption band at 1735 cm.$^{-1}$ indicative of a carbonyl group. The ultraviolet spectrum of actinospectacin shows end absorption and a small amount of absorption in the 290–300 mμ region which varies with the amount of moisture present. The variation of carbonyl absorption in infrared and ultraviolet spectra with the degree of hydration is attributed to hydration of a carbonyl group. The optical rotation of crystalline actinospectacin is slightly positive.

Both the previously mentioned infrared absorption at 1735 cm.$^{-1}$ and chemical data indicate the presence of a ketonic carbonyl in actinospectacin. This ketonic function does not react readily with most of the usual carbonyl reagents, but it does react with thiosemicarbazide to form a thiosemicarbazone. The product of this reaction is the thiosemicarbazone of actinospectacin which has lost a molecule of water, judging from analysis, resulting in an olefinic bond as evidenced by the appearance of a maximum in the ultraviolet region at 287 mμ (ϵ 15,900). Actinospectacin is readily reduced both by sodium borohydride and catalytically with hydrogen to give dihydroactinospectacin demonstrating that only one unsaturated site is reduced. This product no longer has the 1735 cm.$^{-1}$ infrared absorption band under any conditions, indicating reduction of the carbonyl group.

Actinospectacin forms a series of acetyl derivatives of which one (II) is the N,N′-diacetyl derivative. This compound has only two acetyl groups as determined by analysis and is neutral. These facts together with the presence of two N-methyl groups in the antibiotic establish that the nitrogen atoms must be present as methylamino groups. This is confirmed by periodate oxidation of actinospectacin to give, along with other products, two moles of methylamine. A triacetyl derivative of actinospectacin (III) is also readily obtained. This compound is neutral so it must be N,N′,O-triacetylactinospectacin. The infrared spectrum of III has absorption characteristic of ester and amide carbonyls, but it also still has infrared absorption in the hydroxyl region. Consequently, actinospectacin must have two or more hydroxyl groups.

Actinospectacin gave a negative iodoform reaction indicating that the C-methyl is not adjacent to carbonyl or a secondary hydroxyl group. Its action with Tollens reagent is somewhat ambiguous, but it had at best only a very weak reducing action.

Hydrolysis of actinospectacin with boiling 3–6 *N* hydrochloric acid gives a compound, designated actinamine, retaining both basic groups present in the antibiotic. The remainder of the molecule is converted to tarry products. This basic compound was isolated as a crystalline dihydrochloride which is easily converted to a crystalline base (VI) having the molecular formula $C_8H_{18}N_2O_4$ and pK_a' values of 7.2 and 8.9. The infrared spectrum of actinamine has bands at 3440 and 3330 cm.$^{-1}$ indicative of hydroxyl and/or amino groups; but there are no bands attributable to unsaturation of any type. The ultraviolet spectrum shows only end absorption. Actinamine as well as its salts and various derivatives (*vide infra*) is optically inactive in the range 310 to 589 mμ.

Acetylation of actinamine gives a hexaacetyl derivative (VII) which has both amide and ester groups present but no hydroxyl groups as shown by its infrared

(1) Preliminary reports of this work have been published and presented orally. See H. Hoeksema, A. D. Argoudelis, and P. F. Wiley, *J. Am. Chem. Soc.*, **84**, 1514, 3212 (1962); H. Hoeksema, Medicinal Chemistry Symposium, Boulder, Colo., June 18–21, 1962.

(2) The trademark of The Upjohn Co. for actinospectacin is Trobicin.

(3) D. J. Mason, A. Dietz, and R. M. Smith, *Antibiot. Chemotherapy*, **11**, 118 (1961).

(4) M. E. Bergy, T. E. Eble, and R. R. Herr, *ibid.*, **11**, 661 (1961).

(5) A. C. Sinclair and A. F. Winfield, First Interscience Conference on Antimicrobial Agents and Chemotherapy, Oct. 31–Nov. 2, 1961, New York, N. Y.

(6) The molecular formula was previously erroneously reported as $C_{14}H_{26-28}N_2O_7$.[4,5]