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6-Methylenetetracyclines.[1] III. Preparation and Properties[2]

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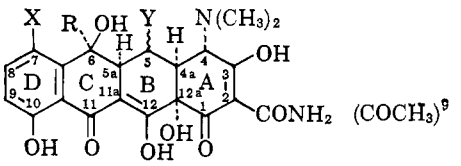
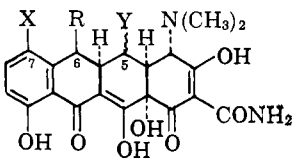
Fermentation tetracyclines (**1**) have been converted to 11a-halotetracycline-6,12-hemiketals (**4**, **5**). Those hemiketals having a methyl group at C-6 undergo exocyclic dehydration in liquid hydrogen fluoride to yield 11a-halo-6-methylenetetracyclines (**7**). The latter are acid stable and suitable substrates for acid-catalyzed substitution reactions. The 11a-halogen is removed under reductive conditions to produce 6-methylenetetracyclines (**10**). In the presence of free-radical catalysts, mercaptans add to the double bond of the 6-methylenetetracyclines. The adducts produced (**19**) are themselves reactive intermediates and have been converted to other derivatives such as sulfoxides (**22**), mercaptan (**23**), and cyclic sulfides (**24**, **25**). Certain aspects of structure–activity in the tetracycline series are considered also.

While there has been much recent research emphasis directed toward the total synthesis of tetracyclines,[3] work has continued apace in the area of chemical modifications of the fermentation-derived tetracyclines (**1**). Thus a rapidly increasing number of new, partially synthetic derivatives possessing broad spectrum antimicrobial activity have been reported in recent years. An important advance in the latter area came with catalytic hydrogenolysis of the fermentation tetracyclines to their 6-deoxy derivatives[10] (*e.g.*, **2a**–**2c**),



1

	X	R	Y
1a, 7-chlorotetracycline[4]	Cl	CH_3	H
1b, 5-hydroxytetracycline[4c,5]	H	CH_3	OH
1c, tetracycline[6]	H	CH_3	H
1d, 7-bromotetracycline[7]	Br	CH_3	H
1e, 7-chloro-6-demethyltetracycline[8]	Cl	H	H
1f, 6-demethyltetracycline[8]	H	H	H



2

	X	R	Y
2a, 6-deoxy-6-demethyltetracycline	H	H	H
2b, β-6-deoxytetracycline	H	CH_3	H
2c, β-6-deoxy-5-hydroxytetracycline	H	CH_3	OH
2d, 7-chloro-6-demethyl-6-deoxytetracycline	Cl	H	H
2e, 7-nitro-6-demethyl-6-deoxytetracycline	NO_2	H	H

compounds with sufficient acid stability to permit the preparation of a variety of active derivatives (*e.g.*, **2d**–**2e**) by way of classical aromatic substitution reactions.[10c,12]

We now wish to describe in detail further advances in the partial synthesis of novel tetracyclines, including the preparation of the 11a-halotetracycline-6,12-hemiketals (**4**, **5**), their exocyclic dehydration to the acid-stable 11a-halo-6-methylenetetracyclines (**7**), and subsequent transformation to 6-methylenetetracycline derivatives (**10**) and to various sulfur-containing tetracyclines (**19**, *et seq.*).[13] The chemistry described is adaptable to the preparation of a broad variety of tetracyclines modified at positions amply demonstrated[2-12] to be outside the area of the molecule essential for antimicrobial activity. The numerous compounds prepared to date have provided a wider base for a more detailed insight into structure–activity relationships in the tetracycline series.

11a-Halotetracycline-6,12-hemiketals.—Interaction of perchloryl fluoride[14] with tetracyclines under basic conditions has resulted in two classes of 11a-fluorine substitution products (**3** and **4**). Simple 11a-fluorotetracyclines (**3**)[2a,10c] are obtained only when the C-6

(1) 6-Methylenetetracycline is a contraction of the name 6-methylene-6-demethyl-6-deoxytetracycline. An alternative name is 6,13-anhydrotetracycline; *cf.* expression **10**.

(2) Much of the material here presented has been the subject of preliminary communications: (a) H. H. Rennhard, R. K. Blackwood, and C. R. Stephens, *J. Am. Chem. Soc.*, **83**, 2774 (1961); (b) R. K. Blackwood, J. J. Beereboom, H. H. Rennhard, M. Schach von Wittenau, and C. R. Stephens, *ibid.*, **83**, 2773 (1961); (c) R. K. Blackwood and C. R. Stephens, *ibid.*, **84**, 4157 (1962). The subject matter has also been presented, in part, at the 137th National Meeting of the American Chemical Society, Cleveland, Ohio, April, 1960, and *in toto* at the Gordon Research Conference on Steroids and Other Natural Products, July, 1962.

(3) See L. H. Conover, K. Butler, J. D. Johnston, J. J. Korst, and R. B. Woodward, *J. Am. Chem. Soc.*, **84**, 3222 (1962), for the total synthesis of 6-demethyl-6-deoxytetracycline and related references.

(4) (a) 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); (b) S. Hirokawa, Y. Okaya, F. M. Lovell, and R. Pepinsky, *Z. Krist.*, **112**, 439 (1959); (c) J. Donohue, J. D. Dunitz, K. N. Trueblood, and M. S. Webster, *J. Am. Chem. Soc.*, **85**, 851 (1963); (d) V. N. Dobrynin, A. J. Gurevich, M. G. Karapetyan, M. N. Kolosov, and M. M. Shemyakin, *Tetrahedron Letters*, **20**, 901 (1962).

(5) (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**, 5455 (1953); (b) Y. Takeuchi and M. J. Buerger, *Proc. Natl. Acad. Sci. U. S.*, **46**, 1366 (1960).

(6) Tetracycline, although first prepared by catalytic hydrogenolysis of 7-chlorotetracycline [L. H. Conover, W. T. Moreland, A. R. English, C. R. Stephens, and F. J. Pilgrim, *J. Am. Chem. Soc.*, **75**, 4622 (1953); J. H. Boothe, J. Morton, J. P. Petisi, R. G. Wilkinson, and J. H. Williams, *ibid.*, **75**, 4621 (1953)], may also be obtained by fermentation [P. P. Minieri, M. C. Firman, A. G. Mistretta, A. Abbey, C. E. Bricker, N. E. Rigler, and H. Sokol, "Antibiotics Annual 1953–1954," Medical Encyclopedia, Inc., New York, N. Y., 1953, p. 81].

(7) P. Sensi, *Farmaco* (Pavia) *Ed. sci.*, **10**, 346 (1955); A. P. Doerschuck, J. R. D. McCormick, J. J. Goodman, S. A. Szumski, J. A. Growich, P. A. Miller, B. A. Bitler, E. R. Jensen, M. A. Petty, and A. S. Phelps, *J. Am. Chem. Soc.*, **78**, 1508 (1956).

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

(9) Three tetracyclines, in which the carboxamide group at the 2-position is replaced by an acetyl group (but otherwise corresponding in structure to **1a**, **1b**, and **1c**), have also been isolated and identified from fermentation broths: F. A. Hochstein, M. Schach von Wittenau, F. W. Tanner, and K. Murai, *ibid.*, **82**, 5934 (1960); F. A. Hochstein and H. W. Miller, *J. Org. Chem.*, **27**, 2525 (1962).

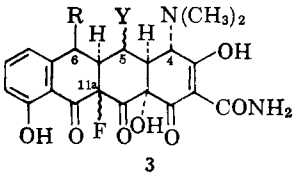
(10) (a) C. R. Stephens, K. Murai, H. H. Rennhard, L. H. Conover, and K. J. Brunings, *J. Am. Chem. Soc.*, **80**, 5324 (1958); (b) J. R. D. McCormick, E. R. Jensen, P. A. Miller, and A. P. Doerschuk, *ibid.*, **82**, 3381 (1960); (c) C. R. Stephens, J. J. Beereboom, H. H. Rennhard, P. N. Gordon, K. Murai, J. J. Ursprung, R. K. Blackwood, and M. Schach von Wittenau, *ibid.*, **85**, 2643 (1963).

(11) M. Schach von Wittenau, J. J. Beereboom, R. K. Blackwood, and C. R. Stephens, *ibid.*, **84**, 2647 (1962).

(12) (a) J. J. Beereboom, J. J. Ursprung, H. H. Rennhard, and C. R. Stephens, *ibid.*, **82**, 1003 (1960); (b) J. H. Boothe, J. J. Hlavka, J. P. Petisi, and J. L. Spencer, *ibid.*, **82**, 1253 (1960); J. J. Hlavka, A. Schneller, H. Krazinski, and J. H. Boothe, *ibid.*, **84**, 1426 (1962); J. Petisi, J. L. Spencer, J. Hlavka, and J. H. Boothe, *J. Med. Pharm. Chem.*, **5**, 538 (1962).

(13) (a) Use of compounds described herein as intermediates for preparation of α- and β-6-deoxytetracyclines is described in separate publications (ref. 10c and 11). (b) Prof. Muxfeldt (ref. 29) employs the nomenclature "6-*epi*" for the series which we call "β."

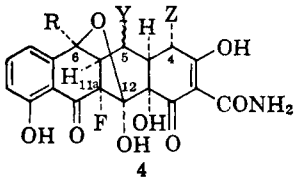
(14) *Cf.* C. E. Inmann, R. E. Oesterling, and E. A. Tyczkowski, *J. Am. Chem. Soc.*, **80**, 6533 (1958); C. E. Inmann, E. A. Tyczkowski, R. E. Oesterling, and F. L. Scott, *Experientia*, **14**, 355 (1958).



3

	R	Y
3a, 11a-fluoro-6-demethyl-6-deoxytetracycline	H	H
3b, 11a-fluoro-β-6-deoxy-5-hydroxytetracycline	CH_3	OH

hydroxyl is *not* present. In cases where the starting tetracycline retains the C-6 hydroxyl group, more involved products are obtained, whose properties clearly indicate them to have the 11a-fluoro-6,12-hemiketal structure (**4**).[15] These derivatives can be reduced to the parent antibiotics and exhibit ultraviolet absorption characteristics typical of 8-hydroxytetralone plus

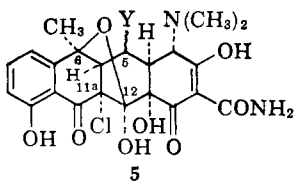


4

	R	Y	Z
4a, 11a-fluorotetracycline-6,12-hemiketal	CH_3	H	$N(CH_3)_2$
4b, 11a-fluoro-5-hydroxytetracycline-6,12-hemiketal	CH_3	OH	$N(CH_3)_2$
4c, 11a-fluoro-6-demethyltetracycline-6,12-hemiketal	H	H	$N(CH_3)_2$
4d, 11a-fluoro-4-dedimethylaminotetracycline-6,12-hemiketal	CH_3	H	H
4e, 11a-fluoro-4-dedimethylamino-5-hydroxytetracycline-6,12-hemiketal	CH_3	OH	H

tetracycline A-ring, in analogy to the simple 11a-fluoro derivatives (**3**). Unlike the latter, however, they show no indication of ketonic absorption below 6 μ, indicating the absence of a free ketone group at C-12. Formation of a 6,12-hemiketal is possible only if the stereochemical requirements shown in expression **4** are met, *i.e.*, 6-oxygen *trans* and 11a-fluorine *cis* to the 5a-hydrogen. This is in agreement with the C-5a–C-6 stereochemistry determined by X-ray crystallography on 7-chlorotetracycline (**1a**),[4b,4c] as well as with that suggested for these positions at the time of the structural elucidation of 5-hydroxytetracycline.[5a] Isolation of compound **4c** provides rigorous experimental evidence that the 6-demethyltetracycline analogs (**1e**, **1f**) have the same stereochemistry at C-6 as do the earlier fermentation tetracyclines.

Interaction of amphoteric tetracycline with N-chlorosuccinimide in a solvent such as 1,2-dimethoxyethane results in the formation of the analogous 11a-chlorotetracycline-6,12-hemiketal (**5a**). The latter shows ultraviolet absorption similar to that of the corresponding fluoro derivative (**4a**). However, some differences in infrared spectra in the two compounds are noted. Thus neither **4a** nor **5a** show carbonyl absorption below



5

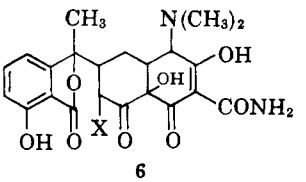
5a, 11a-chlorotetracycline-6,12-hemiketal, Y = H
5b, 11a-chloro-5-hydroxytetracycline-6,12-hemiketal, Y = OH

(15) That the 6-hydroxyl, rather than the 5-hydroxyl, is involved in the specific cases of **4b** and **4e** is inferred from the fact that β-6-deoxy-5-hydroxytetracycline (**2c**, ref. 10) forms the simpler type derivative (**3b**, ref. 2a and 10c)

6 μ in KBr pellet, while the chlorohemiketal alone shows a weak band near 5.8 μ in dioxane solution. These observations suggest that appreciable dissociation to the C-12 ketonic form occurs in the case of the 11a-chlorohemiketal (**5a**), but *not* in the case of the 11a-fluorohemiketal (**4a**).[16] Differences in the chemical behavior of these two compounds can also be interpreted in terms of their differing hemiketal stabilities:

(I) Like *both* the 11a-chloro-6-deoxy[2b,10c] *and* 11a-fluoro-6-deoxy derivatives (**3**) where the 12-ketone is present to activate the halogen, the 11a-chlorohemiketals (**5**) are readily dehalogenated under mild conditions (*e.g.*, sodium hydrosulfite reduction or catalytic hydrogenation at room temperature). This is in sharp contrast to the situation with the 11a-fluorohemiketals, where removal of the halogen is found to be relatively difficult (*e.g.*, 11a-fluorotetracycline hemiketal (**4a**) requires catalytic hydrogenolysis at 5000 p.s.i. and 30–100° for significant reconversion to tetracycline). Presumably the influence of a free 12-ketone is readily available in the case of the chlorohemiketal, but not in the case of the fluorohemiketal.[17]

(II) When heated in methanol–hydrochloric acid the chlorine analog **5a** is cleanly degraded to 11a-chloroisotetracycline (**6a**, a compound characterized by its



6

6a, 11a-chloroisotetracycline, X = Cl
6b, isotetracycline, X = H

composition, spectral properties, and hydrosulfite reduction to isotetracycline, **6b**).[18] Under identical conditions the fluoro derivative **4a** remains unchanged. It is evident that the formation of a compound such as **6a** requires opening of the hemiketal prior to attack of the 6-hydroxyl on the 11-ketone and opening of the 11,11a-bond. Noteworthy is the fact that the 5a,6-elimination reaction, so characteristic of normal tetracyclines, does not occur with these 11a-halo compounds. The theoretical significance of this observation is discussed fully in the section below.

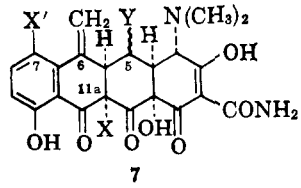
6-Methylenetetracyclines.—In marked contrast to the degradation seen in hot methanol–hydrochloric acid (**5a** → **6a**) is the dehydration of 11a-chlorotetracycline-6,12-hemiketal in liquid hydrogen fluoride.[19] In spite of the fact that the hemiketal retains the *trans* relationship of 5a-hydrogen and 6-oxygen, the classical 5a,6-endocyclic dehydration of the tetracyclines is not observed. Instead, clean and rapid exocyclic dehydration occurs to yield the 11a-chloro-6-methylene derivative

(16) Since completion and preliminary communication of our own studies (ref. 2a and 2b), we have examined the 11a-bromodedimethylaminotetracycline of A. Green, R. G. Wilkinson, and J. H. Boothe [*J. Am. Chem. Soc.*, **82**, 3946 (1960)]. We repeated both of their preparations. The method employing N-bromosuccinimide in chloroform gave product which showed infrared spectral behavior (in KBr pellet and dioxane solution) analogous to that of **5a**. In contrast, the method employing bromine in acetic acid (slightly modified, *cf.* Experimental section) gave a product possessing carbonyl absorption near 5.8 μ in both KBr pellet and dioxane solution. Recrystallization of the latter product from warm chloroform gave material identical with the former product. We would therefore conclude that the product has simply crystallized in one instance as the hemiketal and in the other as the open ketone form.

(17) The important role of the 12-ketone in promoting reduction is further emphasized by the fact that **6a** is readily dechlorinated by sodium hydrosulfite, while **13** is stable under similar conditions.

(18) J. H. Booth, J. Morton, J. P. Petisi, R. G. Wilkinson, and J. H. Williams, "Antibiotics Annual, 1953–1954," Medical Encyclopedia, Inc., New York, N. Y., 1953, p. 47.

(19) These two reactions are not interrelated as evidenced by the stability of compound **6a** in liquid hydrogen fluoride and by the stability of the HF product **7a** in hot methanol–hydrochloric acid.

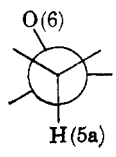


7

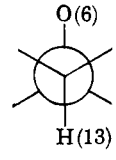
	X	X'	Y
7a, 11a-chloro-6-methylenetetracycline	Cl	H	H
7b, 11a-fluoro-6-methylenetetracycline	F	H	H
7c, 11a-chloro-5-hydroxy-6-methylenetetracycline	Cl	H	OH
7d, 7,11a-dichloro-5-hydroxy-6-methylenetetracycline	Cl	Cl	OH
7e, 11a-fluoro-5-hydroxy-6-methylenetetracycline	F	H	OH

7a. Structural assignment is based on composition, formation of formaldehyde on ozonolysis,[20] n.m.r. studies (no C-methyl signal, a doublet at τ = 4.4 assigned to the methylene group),[21] ultraviolet absorption (a bathochromic shift in the long wave length maxima is consistent with extended conjugation in the 8-hydroxytetralone chromophore), infrared absorption (a band at 5.7 μ assigned to the 12-ketone), and subsequent reactions.

A possible reason for exocyclic rather than endocyclic dehydration may be educed from an examination of conformational models. Figures **8a** and **8b** show Newman projections along the C-5a–C-6 and C-6–C-13-bonds *in the hemiketal*. It is to be noted that the 6-oxy-

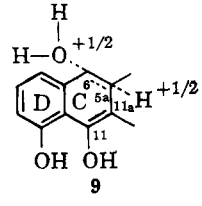


8a



8b

gen and 5a-hydrogen cannot exist in the perfectly planar, *trans* relationship preferred for elimination. In contrast, any one of the hydrogens on the C-6-methyl group (designated C-13) may readily assume the preferred conformation. However, since these differences do not hold when the hemiketal opens, and since facile opening of the chloro hemiketal has been indicated from studies described above, a second argument to explain exocyclic dehydration appears more reasonable. Figure **9** shows a pro-



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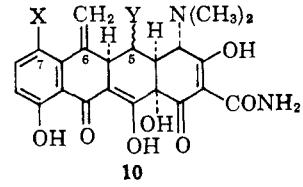
posed transition state for normal endocyclic dehydration. It seems probable that a critical feature of this transition state is a C-11–C-11a double bond, with formation of an aromatic C-ring as an important driving force. It is obvious that such a bond cannot exist with a blocking group present at the 11a-position. 11a-Fluorotetracycline-6,12-hemiketal (**4a**) is similarly dehydrated in hydrogen fluoride to a 6-methylene derivative (**7b**). However, the latter reaction proceeds at a much slower rate, suggesting that hemiketal opening precedes dehydration and is rate controlling. A further example of the lack of tendency toward 5a,6-elimination of water in these compounds is seen in the observation that the corresponding 11a-fluoro-6-demethyl compound **4c** (where only endocyclic elimination is possible) is completely stable under similar conditions.

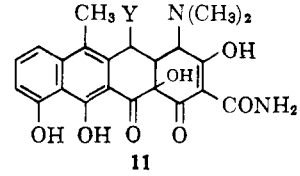
11a-Chloro-6-methylenetetracycline (**7a**) is readily reduced to the fully biologically active 6-methylene-



10

	X	Y
10a, 6-methylenetetracycline	H	H
10b, 6-methylene-5-hydroxytetracycline	H	OH
10c, 7-chloro-6-methylene-5-hydroxytetracycline	Cl	OH

tetracycline (**10a**).[22] Structural assignment is based on composition, acid rearrangement to known 5a,6-anhydrotetracycline[4a] (**11a**), ozonolysis,[20] and catalytic

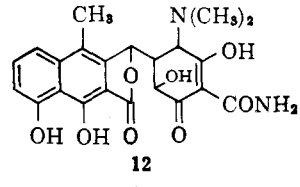


11

11a, 5a,6-anhydrotetracycline, Y = H
11b, 5a,6-anhydro-5-hydroxytetracycline, Y = OH

reduction to β- and α-6-deoxytetracycline (**2b** and its 6-epimer, **20a**, respectively) reported elsewhere.[10c,11] When ultraviolet spectra of **10a** and 6-demethyl-6-deoxytetracycline (**2a**) are compared, they are found to differ primarily in the short wave length region, where the methylene analog exhibits the enhanced, broadened absorption we have found to be characteristic of this type compound. When infrared spectra of the same two compounds are compared in chloroform solution, only the methylene derivative shows a band at 11.1 μ, consistent with a methylene function.

By application of the same three-step reaction sequence (chlorination, dehydration, and reduction), 5-hydroxytetracycline (**1b**) is converted, *via* intermediates **5b** and **7c**, into the corresponding methylene derivative (**10b**). The spectral and chemical properties of the intermediates and final product parallel those of the corresponding tetracycline analogs. The structure of **10b** is firmly established by ozonolysis,[20] catalytic hydrogenation to the α- and β-6-deoxy-5-hydroxytetracyclines[10c,11,13b] (**20b** and **2c**), and acid degradation to known α- and β-apoterramycins (**12**),[5a] formed *via* the 5a,6-anhydro intermediate (**11b**).[5a] The latter is unsta-



12

ble under the relatively vigorous acid conditions needed to rearrange the exocyclic double bond into the ring.[23]

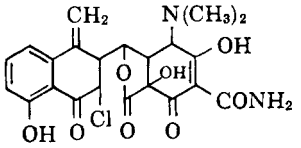
(20) Formaldehyde was isolated in 19–23% yield as its dimedone adduct. Blank reactions with **1b** and **5a** gave no significant quantity of formaldehyde.

(21) C-Methyl analyses on **5a**, together with appropriate blanks, also confirm the methylene structure.

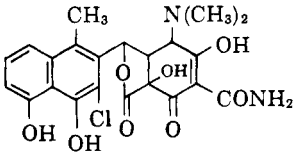
(22) This reduction also occurs *in vivo* in various animal species, including man. Thus 11a-chloro-6-methylenetetracycline (**7a**), while showing only a low order of activity *in vitro*, is fully as active *in vivo* as the 11a-dechlorinated material (**10a**).

(23) Using procedures related to those employed earlier on tetracycline [*cf.* 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)] it is possible to convert the 6-methylene-5-hydroxy derivative (**10b**) to its 4-dedimethylamino analog *via* zinc reduction of the methiodide (*cf.* Experimental). In marked contrast to the situation with the parent 5-hydroxytetracycline (**1b**), no tetramethylammonium iodide is noted in the reaction of methyl iodide with **10b** (*cf.* L. H. Conover, "Special Publication No. 5," The Chemical Society, London, 1956, p. 72, *et seq.*).

Differences are to be noted in the synthetic sequence as applied to tetracycline and to 5-hydroxytetracycline. Thus, dehydration of the hemiketal to produce the 11a-chloro-6-methylene intermediate (**7c**) proceeds more slowly than in the tetracycline case, paralleling the greater acid stability of 5-hydroxytetracycline over tetracycline.[24] Secondly, this intermediate undergoes a facile degradation (for example in an aqueous solvent as the pH is raised into the isoelectric region) to form a lactone (**13**).[25] The assignment of structure to this lactone is based on a number of arguments. Thus, the ultraviolet spectrum shows retention of the methylene-8-hydroxytetralone and A-ring chromophores. The infrared spectrum shows a band at 5.6 μ assigned to the 5-membered lactone. That the lactone is attached to 12a, rather than 11a, is indicated by a further degradation which occurs on refluxing **13** in dimethylformamide–acetone. A crude compound, presumed to have structure **14**, is formed. The latter shows the typical ultraviolet properties of 1,8-dihydroxynaphthalene plus tetracycline A-ring, analyzes for one mole of organic chlorine, and finally, retains the 5.6 μ lactone band. If the lactone were attached to the 11a, rather than the 12a position, a 1,8-dihydroxynaphthalene compound could not form without loss of chlorine or of the lactone. Furthermore, if the lactone were attached at 11a rather than 12a in the final degradation product, it should have a characteristic apoterramycin-type chromophore.[5a]

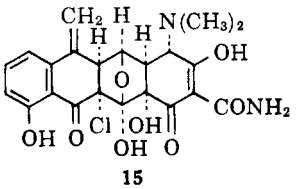


13



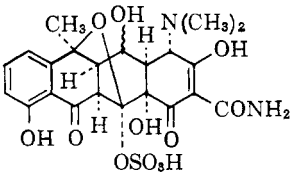
14

The facility with which the lactone **13** is formed from the chloromethylene compound **7c** bears on the question of C-5 stereochemistry in 5-hydroxytetracycline (**1b**). Since the lactone is formed cleanly, without evidence of an intermediate acid, and since the tetracycline analog **7a**, lacking a 5-hydroxyl, is stable under similar conditions, it is concluded that the conversion **7c** → **13** occurs *via* a hemiketal intermediate such as **15**. Model studies indicate that if the 5-hydroxyl were *cis* to the 4a- and 5a-hydrogens in such an intermediate, very large 1,3-type interactions (*i.e.*, A-ring with the C,D-ring system) would be involved. In contrast, with the 5-hydroxyl *trans* to the 4a- and 5a-hydrogens, minimal 1,3-interactions (*i.e.*, 4a-hydrogen with 5a-hydrogen, 11a-chlorine with 12a-hydroxyl) are permitted. Like the recent X-ray evidence of Donohue, Dunitz, Trueblood, and Webster,[4c] this does not allow an unequivocal conclusion to be drawn regarding C-5 stereochemistry in 5-hydroxytetracycline (**1b**). However, it does provide a further indication for a *trans* relationship of 5-hydroxyl to 4a- and 5a-hydrogens, in contrast to the X-ray stereochemistry depicted earlier by Takeuchi and Buerger.[5b]

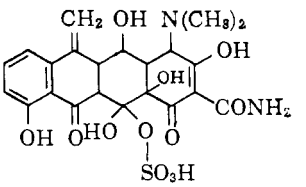


15

That a halogen as blocking group at the 11a-position is not an essential feature for exocyclic elimination is demonstrated by an alternative preparation of 6-methylene-5-hydroxytetracycline (**10b**). Thus, treatment of 5-hydroxytetracycline (**1b**) with pyridine–sulfur trioxide produces a sulfuric acid ester presumed, on the basis of spectra, methanolysis, and dehydration studies, to have structure **16**. Treatment of this ester with anhydrous hydrogen fluoride converts it directly to 6-methylene-5-hydroxytetracycline, presumably *via* intermediate **17** which should readily eliminate sulfuric acid. Because the final product is quite unstable in hydrogen fluoride, the yields of 6-methylene-5-hydroxytetracycline are relatively low by this process.

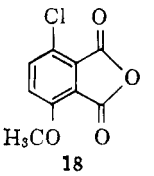


16



17

The 6-methylenetetracyclines (**10**) possess an acid stability intermediate between that of tetracycline(**1c**) and that of the 6-deoxytetracyclines (**2**). However, because the 11a-fluoro- and 11a-chloro-6-methylenetetracyclines (**7**) resemble the latter in their acid stability, they are suitable substrates for reactions catalyzed by strong acid. An example of aromatic substitution in the methylene series is seen in the preparation of 7-chloro-6-methylene-5-hydroxytetracycline (**10**). Treatment of the 11a-chloro-6-methylene-5-hydroxytetracycline (**7c**, conveniently formed *in situ* from the hemiketal precursor, **5b**) with N-chlorosuccinimide in anhydrous hydrogen fluoride produces the 7,11a-dichloro intermediate (**7d**). The latter shows the typical spectral properties of starting material **7c**, except for a further bathochromic shift in the short wave length ultraviolet maximum due to the aromatic chlorine. On reduction with sodium hydrosulfite, the 11a-chlorine is removed to yield the desired, biologically active product **10c**. The 7-chloro-6-methylene structure is assigned on the basis of ultraviolet absorption (related to both those of **10b** and **2d**), n.m.r. spectrum (which shows retention of the methylene group and only two aromatic hydrogens), and exhaustive methylation–oxidation to 6-chloro-3-methoxyphthalic anhydride (**18**).[26]



18

Mercaptan Adducts.—The 6-methylenetetracyclines (**10**) react with mercaptans to produce adducts of structure **19**. Exemplary of the 13-alkyl-, aryl-, aralkyl-, and acyl-α-6-deoxytetracyclines produced by this process are compounds **19a–19g**.[27] The reaction is a typical free-radical addition of mercaptan to olefinic double bond,[28] being catalyzed by oxygen, peroxide, or light. In practice, 2,2′-azo-bis-(2-methylpropionitrile) is a most useful catalyst for the reaction, since oxidation at sulfur is not encountered (as is the case with peroxide or air catalysis) and there is no difficulty in repro-

(24) It has been our general experience that tetracycline derivatives are less stable to acid than the corresponding 5-hydroxy- or 7-chlorotetracycline derivatives. For example, the following sequence of acid stability is observed **1a** > **1c**, **1b** > **1c**, **10c** > **10b** > **10a**.

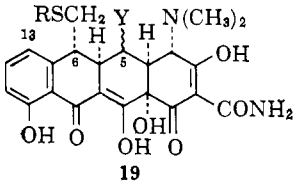
(25) The instability of **7b** presumably accounts for the fact that, unlike the simpler analog **7a**, it does not show the *in vivo* activity found with dechlorinated products **10a** and **10b**; *cf.* footnote 22.

(26) Previously isolated by similar treatment of 7-chlorotetracycline; *cf.* S. Kushner, J. H. Boothe, J. Morton, J. Petisi, and J. H. Williams, *J. Am. Chem. Soc.*, **74**, 3710 (1952).

(27) Other mercaptans successfully employed include R = CH_3, *n*-C_4H_9, $HOOCCH_2$, $C_2H_5OOCCH_2$, $H_2NCH_2CH_2$, and $HOOCCH(NH_2)CH_2$.

(28) See C. Walling, "Free Radicals in Solution," John Wiley and Sons, Inc., New York, N. Y., 1957, pp. 313–326, for a discussion of this reaction.

ducibility on scale up (as tends to be the case with light catalysis). Solvent for the reaction is not critical, the mercaptan itself being employed where suitable. Temperature may be varied over a wide range, with reactions conveniently followed by paper chromatography.

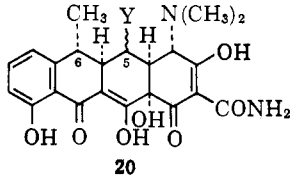


19

α-6-Deoxytetracycline[13b]	R	Y
19a, 13-phenylmercapto-	C_6H_5	H
19b, 13-phenylmercapto-5-hydroxy-	C_6H_5	OH
19c, 13-benzylmercapto-	$C_6H_5CH_2$	H
19d, 13-benzylmercapto-5-hydroxy-	$C_6H_5CH_2$	OH
19e, 13-(2-hydroxyethylmercapto)-5-hydroxy-	$HOCH_2CH_2$	OH
19f, 13-acetylmercapto-	CH_3CO	H
19g, 13-acetylmercapto-5-hydroxy-	CH_3CO	OH

As might be expected on steric grounds, mercaptans add most readily to 6-methylenetetracycline (**10a**). The 5-hydroxy analog (**10b**) is of intermediate activity while the 7-chloro-5-hydroxy compound (**10c**) is very unreactive toward mercaptans. The principal side reactions encountered involve initial rearrangement of the double bond into the ring to form 5a,6-anhydro compounds (**11**). It is our impression that the amount of side reaction increases with (1) decreasing acid stability of the methylene compound,[24] (2) increasing acidity of the mercaptan, and (3) decreasing reactivity of the mercaptan.

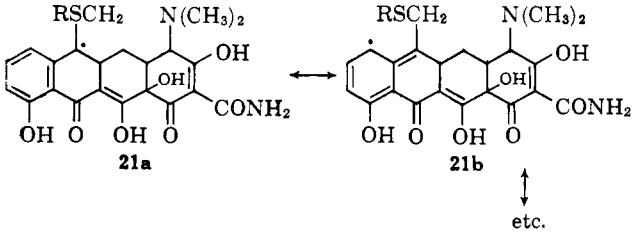
That the products of the reaction arise from addition across the double bond is evident from analyses, ultraviolet spectra (there is no longer the extended BCD-ring chromophore of the 6-methylenetetracyclines), and the enhancement of acid stability to a level typical of that of the 6-deoxytetracyclines.[10,11] That the direction of addition is anti-Markovnikov is inferred from reaction type,[28] and confirmed by C-methyl analyses and n.m.r. spectra which show lack of C-methyl groups. Stereochemistry at C-6 is assigned on the basis of Raney nickel desulfurization to the α-6-deoxytetracyclines (**20**),[11] for which independent stereochemical arguments have been presented.[10c,11,29] Furthermore, the addition reaction is completely stereospecific; it is reasonable



20

20a, α-6-deoxytetracycline, Y = H
20b, α-6-deoxy-5-hydroxytetracycline, Y = OH

that a stabilized intermediate free radical (**21**) would lead to what model studies indicate to be the thermody-

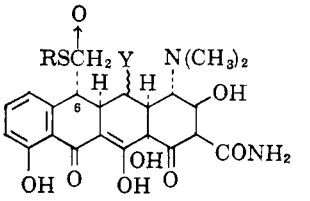


21a 21b

namically stable product, *i.e.*, with the large $RSCH_2$– group pseudoequatorial.

(29) H. Muxfeldt, *Angew. Chem. Intern. Ed. Engl.*, **1**, 372 (1962).

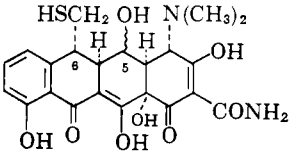
The tetracycline thio compounds undergo a variety of reactions typical of sulfur chemistry. Thus, under mild conditions the sulfides are oxidized to the corresponding sulfoxides (*e.g.*, **22a**, **22b**). Hydrolysis of acyl



22

22a, 13-phenylmercapto-α-6-deoxytetracycline S-oxide, Y = H
22b, 13-benzylmercapto-α-6-deoxytetracycline S-oxide, Y = H

compounds is typified by the conversion, in concentrated hydrochloric acid, of 13-acetylmercapto-α-6-deoxytetracycline (**19g**) into the mercaptan (**23**). The lat-

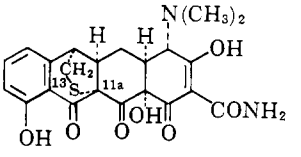


23

13-mercapto-5-hydroxy-α-6-deoxytetracycline

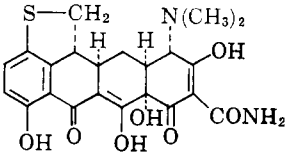
ter is characterized by its composition and typical mercaptan color reaction with sodium nitroprusside, not seen with the starting material (**19g**).

A reaction which may be less typical of sulfur chemistry is the conversion, in concentrated hydrochloric acid, of the benzyl S-oxide (**22b**) into two compounds for which we propose structures **24** and **25**. Both compounds



24

11a,13-epithio-α-6-deoxytetracycline



25

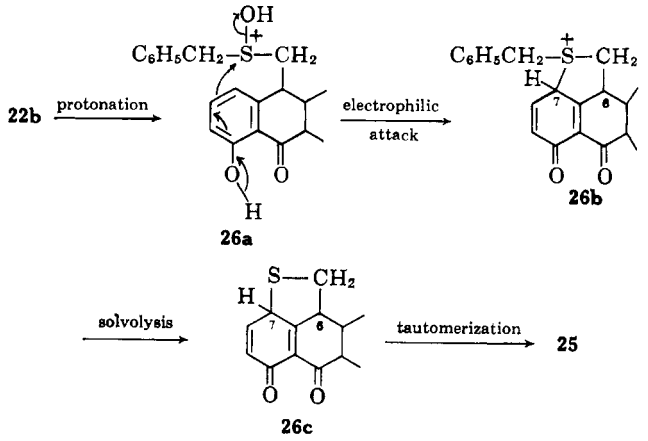
7,13-epithio-α-6-deoxytetracycline

have analyses which clearly indicate loss of the benzyl group. Compound **24** shows the typical spectral properties of 11a-blocked tetracyclines retaining the 12-ketone. Like 11a-chloro-6-methylenetetracycline (**7a**), it does not show appreciable antimicrobial activity *in vitro*, but is active *in vivo*,[30] suggesting no other drastic alteration in the tetracycline molecule. Compound **25** gives a negative sodium nitroprusside test, indicating lack of a mercapto group. It exhibits typical, broad spectrum tetracycline-like activity,[30] suggesting changes only along the upper periphery of the molecule. Its ultraviolet spectrum in methanol–sodium hydroxide does not differ greatly from that of an ordinary tetracycline. However, its ultraviolet absorption in acid shows pronounced splitting of the long wave length maximum, clearly indicating involvement of the BCD-ring chromophore.[31] The following mechanism reasonably accounts for the formation of compound **25**. Formation of **24** would be analogous, except for electrophilic attack at the 11a-, rather than the 7-position.

Structure–Activity.—An activity comparison, based on *Klebsiella pneumonia* turbimetric bioassay proce-

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

(31) A modified tautomeric form in acid, such as **26c**, which would relieve strain in the 5-membered sulfur ring, is suggested to account for these unusual spectral properties.



dure,[32] of new derivatives reported herein along with some earlier tetracyclines is shown in Table I. Particularly wide variation in activity is to be noted among the sulfur analogs. Clearly, these gross differences in

TABLE I

In Vitro ACTIVITY OF TETRACYCLINES AGAINST *Klebsiella pneumoniae*

Compound	Substituents C-7	C-6α	C-6β	C-5	Bioassay[a]
1b	H	CH_3	OH	OH	1000
1c	H	CH_3	OH	H	1000
10a	H	CH_2		H	1200
10b	H	CH_2		OH	2300
10c	Cl	CH_2		OH	6300
19a	H	$C_6H_5SCH_2$	H	H	10
19b	H	$C_6H_5SCH_2$	H	OH	200
19c	H	$C_6H_5CH_2SCH_2$	H	H	10
19d	H	$C_6H_5CH_2SCH_2$	H	OH	260
19e	H	$HO(CH_2)_2SCH_2$	H	OH	60
19f	H	$CH_3C(=O)SCH_2$	H	H	600
19g	H	$CH_3C(=O)SCH_2$	H	OH	400
20a	H	CH_3	H	H	700
20b	H	CH_3	H	OH	1400
22a	H	$C_6H_5\overset{O}{\overset{\uparrow}{S}}CH_2$	H	H	400
22b	H	$C_6H_5CH_2\overset{O}{\overset{\uparrow}{S}}CH_2$	H	H	240
23	H	$HSCH_2$	H	OH	60
24	S—CH_2		H	H	1300

[a] 5-Hydroxytetracycline standard (1000 γ/mg.); all values corrected to the base form.

activity go beyond any simple structure–activity relationship, since earlier modifications at the 6-position have given rise to relatively minor changes in activity.[2b,8,10,11] While steric, conformational, and/or electronic effects are not to be completely excluded, it appears that over-all polarity of the molecule plays a critical role in determining activity variation among the sulfur tetracyclines. Thus the phenyl and benzyl mercaptan adducts of 6-methylenetetracycline (**19a** and **19c**), possessing highly lipophilic character, show depressed activity, particularly against gram-negative bacteria typified by *Klebsiella pneumonia*. When the latter compounds are converted to the more polar sulfoxides (**22a**, **22b**) they regain much of their lost activity. In line with this viewpoint, the corresponding sulfide derivatives (**22c**, **22d**) in the more polar 5-hydroxytetracycline series show much less depression in activity.[33,34]

(32) R. C. Kersey, *J. Am. Pharm. Assoc.*, **39**, 252 (1950).

(33) Preliminary evidence, based upon crude mercaptan adducts and sulfoxides, would indicate that an optimum polarity range exists such that a high degree of polarity can be equally detrimental to activity. The generally depressed activity of the mercaptan adducts may well result from steric interaction of groups at C-13 with the 11,12-β-diketone system.

(34) The importance of lipophilicity as a factor in pharmacodynamic characteristics of the tetracyclines has been discussed recently: M. Schach von Wittenau and R. Yeary, *J. Pharm. Expti. Therap.*, **140**, 258 (1963).

Experimental

Fluorination Experiments.—All fluorinations with perchloryl fluoride were carried out in a three-neck round-bottom flask equipped with stirrer, and gas inlet and outlet tubes including bubble counters by which the rate of absorption could be checked. The tetracyclines were dissolved in an atmosphere of nitrogen in order to prevent degradation, and after the reaction, excess perchloryl fluoride was swept out with a stream of nitrogen. In the cases where water was used as the solvent, the flask was equipped with a pH meter probe assembly. All fluorinations were carried out at 0° (ice bath).

11a-Fluorotetracycline-6,12-hemiketal (4a).—Amphoteric tetracycline (2.22 g., 5 mmoles) was dissolved in 95 ml. of water and 5 ml. of 2 *N* sodium hydroxide solution. Perchloryl fluoride was passed in. After 90 min., precipitation started at pH 8. After 140 min., the pH was 7.7 and remained constant. The precipitate was filtered off, washed with water, and dried *in vacuo* over phosphorus pentoxide; yield 1.15 g. (48%) of nearly white crystalline material; $\lambda_{max}^{MeOH-0.01\ N\ HCl}$ 265, 336 mμ; log ε 4.45, 3.71; infrared (KBr and THF): no ketonic absorption below 6 μ.

Anal. Calcd. for $C_{22}H_{23}N_2O_8F\cdot H_2O$: C, 54.95; H, 5.20; N, 5.83. Found: C, 54.97; H, 5.19; N, 5.85.

Hydrogenolysis of **4a** was carried out by dissolving 1 g. in 75 ml. absolute tetrahydrofuran and hydrogenating in the presence of 500 mg. of 5% Pd–C at 5000 p.s.i., room temperature for 3 hr. The catalyst was filtered off and the filtrate evaporated *in vacuo*. The residue was triturated with ether and dried. It contained about 26% tetracycline according to bioassay, ultraviolet spectrum, and paper chromatography. Acid treatment (30 min. reflux in 2 *N* methanolic hydrochloric acid) gave anhydrotetracycline (ultraviolet and paper chromatographic evidence).

Zinc reduction of **4a** also produced a mixture of tetracycline and anhydrotetracycline. Compound **4a** (1 g.) was dissolved in 25 ml. of 0.2 *N* HCl. Zinc dust (60 mg.) was added and the mixture stirred vigorously for 15 min. at room temperature. The zinc was removed by filtration and the mother liquor extracted six times with 10-ml. portions of butanol. The combined butanol extracts were combined and evaporated to dryness *in vacuo* to yield 730 mg. of crude product containing *ca.* 60% tetracycline and a lesser amount of 5a,6-anhydrotetracycline (paper chromatographic, bioassay, and ultraviolet absorption evidence). Pure 5a,6-anhydrotetracycline hydrochloride was obtained from this mixture by crystallization from butanol–concentrated HCl.

When 100 mg. of **4a** was refluxed for 30 min. in 10 ml. of 4 *N* aq. methanolic HCl (1 part concentrated HCl, 2 parts methanol), there was no change in ultraviolet spectrum, or in paper chromatographic behavior.

11a-Fluoro-5-hydroxytetracycline-6,12-hemiketal (4b).—Amphoteric, anhydrous 5-hydroxytetracycline (6.9 g., 15 mmoles) was dissolved in 285 ml. methanol and 16.5 ml. of 1 *N* methanolic sodium methoxide. Perchloryl fluoride was passed into the yellow suspension of the sodium salt, which dissolved after 5 min. After 20 min. a heavy precipitate started to form. The flow rate of the perchloryl fluoride was reduced to produce an absorption rate of about 90%. Absorption stopped after 80 min., and the pH of the reaction mixture was *ca.* 5 (diluted with an equal volume of water). The crystalline precipitate was filtered off, washed with methanol, and dried *in vacuo;* yield 5.12 g. (66%) of pale yellow crystals; $\lambda_{max}^{MeOH-0.01\ N\ HCl}$ 265, 335 mμ; log ε 4.41, 3.72; λ_{max}^{KBr} no ketonic absorption below 6 μ.

Anal. Calcd. for $C_{22}H_{23}N_2O_9F\cdot 2H_2O$: C, 51.40; H, 5.25; N, 5.45. Found: C, 51.23; H, 5.29; N, 5.73.

11a-Fluoro-6-demethyltetracycline-6,12-hemiketal (4c).—Amphoteric 6-deoxytetracycline (2.15 g., 5 mmoles) was dissolved in 90 ml. of water and 10 ml. of *N* sodium hydroxide solution. Perchloryl fluoride was passed in for 210 min., after which time the pH had changed from 12.1 to 6.8. A small amount of crystalline solid was filtered off, washed, and dried (210 mg.). The bulk of the product was isolated by freeze drying and extraction with methanol, which yielded an additional 1.65 g. of product; $\lambda_{max}^{MeOH-0.01\ N\ HCl}$ 264, 337 mμ; log ε 4.40, 3.67; λ_{max}^{KBr} no ketonic absorption below 6 μ.

Anal. Calcd. for $C_{21}H_{21}N_2O_8F\cdot H_2O$: C, 54.00; H, 4.93; N, 6.04. Found: C, 53.98; H, 4.91; N, 5.80.

11a-Fluoro-4-dedimethylaminotetracycline-6,12-hemiketal (4d).—Dedimethylaminotetracycline (2 g., 5 mmoles) was suspended in 100 ml. of methanol and 6.1 ml. of 0.9 *N* methanolic sodium methoxide. The suspension of the sodium salt was treated with perchloryl fluoride for 25 min., after which time the solid had dissolved and the solution became dark. The solution was evaporated to dryness. Trituration of the residue with water gave

0.74 g. (34%) of crystalline material, which was recrystallized from methanol (long felty needles); $\lambda_{max}^{MeOH-0.01\ N\ HCl}$ 265, 345 mμ; log ε 4.29, 3.57; λ_{max}^{KBr} no ketonic absorption below 6 μ; acid stability: 10 mg. of **4d** was refluxed 30 min. in 3 *N* anhydrous methanolic hydrochloric acid; there was no change in ultraviolet absorption.

Anal. Calcd. for $C_{20}H_{18}NO_8F \cdot 0.5CH_3OH$: C, 56.80; H, 4.45; N, 3.28. Found: C, 56.80; H, 4.44; N, 3.65.

11a-Fluoro-4-dedimethylamino-5-hydroxytetracycline-6,12-hemiketal (**4e**).—4-Dedimethylamino-5-hydroxytetracycline (2.08 g., 5 mmoles) was dissolved in 100 ml. of methanol; 10.5 ml. (2.1 equiv.) of *N* methanolic sodium methoxide was added and the mixture treated with perchloryl fluoride for 2.5 hr. A crystalline precipitate formed and the pH was 6.6. The product was filtered off (0.94 g., 42%). The filtrate was evaporated to dryness and triturated with water to yield an additional 0.76 g. (34%) of crystalline product, which was recrystallized from methanol; $\lambda_{max}^{MeOH-0.01\ N\ HCl}$ 263, 335 mμ, log ε 4.41, 3.72; λ_{max}^{KBr} no ketonic absorption below 6 μ.

Anal. Calcd. for $C_{20}H_{18}NO_9F \cdot H_2O$: C, 56.74; H, 4.71; N, 3.31. Found: C, 56.79; H, 4.85; N, 3.34.

11a-Chlorotetracycline-6,12-hemiketal (**5a**).—Anhydrous amphoteric tetracycline (8.8 g., recrystallized from refluxing toluene) was dissolved in 90 ml. of 1,2-dimethoxyethane. The solution was clarified by filtration with a 10-ml. wash by the same solvent. To the filtrate there was added 3.2 g. of powdered N-chlorosuccinimide and the mixture permitted to stir for 10 min. at room temperature. During this time a precipitate formed which redissolved on addition of 100 ml. of water. The product crystallized over a period of 10 min. and was recovered by filtration. The product was washed well with water and then reslurried in acetone and refiltered with acetone wash. The product was dried *in vacuo* at room temperature[35]; yield 4.0 g. (41%) of white crystalline product with the spectral properties: $\lambda_{max}^{MeOH-0.01\ N\ HCl}$ 258 and 343 mμ; log ε 4.33 and 3.60; λ_{max}^{KBr} no bands in the 5 to 6-μ region; $\lambda_{max}^{dioxane}$ 5.8 μ.

Anal. Calcd. for $C_{22}H_{24}N_2O_8Cl \cdot 0.5H_2O$: C, 54.2; H, 5.0; N, 5.7; Cl, 7.3. Found: C, 54.3; H, 5.3; N, 5.7; Cl, 6.7.

11a-Chlorotetracycline-6,12-hemiketal does not show ultraviolet fluorescent zones on paper chromatography nor a bioassay. However, a solution (1 mg./ml. in 50% aqueous methanol) treated with excess sodium hydrosulfite shows a strong tetracycline zone on paper chromatography and a bioassay which indicates 50–60% regeneration of tetracycline.

Hydrogenation of 11a-chlorotetracycline-6,12-hemiketal (960 mg. in 50 ml. methanol) with 200 mg. of 5% Rh–C for 2 hr. at atmospheric pressure and room temperature proceeded readily (1 hr.) to provide a mixture of approximately equal parts tetracycline and 5a,6-anhydrotetracycline.

11a-Chloro-5-hydroxytetracycline-6,12-hemiketal (**5b**).—5-Hydroxytetracycline hydrochloride (10 g.) was dissolved in 200 ml. of 50% aqueous 1,2-dimethoxyethane, previously chilled to 5°, with the aid of 3.5 ml. of triethylamine. There was immediately added 4.4 g. of powdered N-chlorosuccinimide. The temperature of the reaction mixture, which was maintained in an ice–water bath, rose to 12° during these processes. The mixture, from which the product began to crystallize rapidly, was stirred for 10 min. in the ice bath and then filtered with sufficient acetone wash to remove dark colored impurities; yield of aid-dried product, 5.0 g. (50%).

An analytical sample was prepared by recrystallization from methanol–hydrochloric acid; spectral properties: $\lambda_{max}^{MeOH-0.01\ N\ HCl}$ 265 and 338 mμ; log ε 4.39 and 3.67; λ_{max}^{KBr} no bands 5 to 6 μ.

Anal. Calcd. for $C_{22}H_{23}O_9N_2 \cdot CH_3OH \cdot HCl$: C, 49.0; H, 5.0; N, 5.0; Cl^-, 6.3; Cl, 12.6; CH_3O, 5.5. Found: C, 49.0; H, 5.0; N, 4.8; Cl^-, 6.3; Cl, 12.7; CH_3O, 5.4.

As with **5a**, the parent antibiotic may be regenerated by reduction with sodium hydrosulfite.

11a-Chloroisotetracycline (**6a**).—11a-Chlorotetracycline-6,12-hemiketal (**5b**, 2.5 g.) was refluxed in 125 ml. of methanol and 62.5 ml. of concentrated HCl for 30 min. The solution was filtered and the filtrate taken to dryness on the rotating evaporator *in vacuo*. The yield of crystalline product, which ultraviolet and papergram assays indicated to be very clean, was 2.4 g. A sample for analysis was prepared by recrystallization from a mixture of hot nitromethane (nonsolvent) and methanol (solvent): λ_{max}^{KBr} 5.65 and 5.73 μ; $\lambda_{max}^{MeOH-0.01\ N\ HCl}$ 240 and 272 mμ, log ε 4.11 and 4.99.

Anal. Calcd. for $C_{22}H_{23}N_2O_8Cl \cdot HCl$: C, 51.3; H, 4.7; N, 5.4; Cl, 13.8; Cl^-, 6.9. Found: C, 50.8; H, 4.8; N, 5.4; Cl, 13.6; Cl^-, 6.9.

11a-Chloroisotetracycline hydrochloride (100 mg.) was converted to known isotetracycline[18] by dissolving in 7 ml. of water, adding 100 mg. of sodium hydrosulfite, and stirring for 30 min. at room temperature. Isotetracycline (40 mg. of the amphoteric form) was recovered by filtration and identified by spectral and chromatographic techniques.

Both isotetracycline and 11a-chloroisotetracycline were recovered unchanged after standing in liquid hydrogen fluoride for 10 min.

11a-Chloro-6-methylenetetracycline (**7a**).—Anhydrous hydrogen fluoride (125 ml.) was added, from an inverted "lecture bottle" size cylinder, to a 500-ml. polyethylene bottle maintained in an ice bath and stirred by a magnetic stirring bar (Teflon covered). 11a-Chlorotetracycline-6,12-hemiketal (16 g.) was added portionwise. After 15 min. the ice bath was removed and the HF boiled off under a stream of nitrogen and a filterable crystalline solid obtained by stirring the residue with 450 ml. of ether. The yield of crude hydrofluoride salt was 16.4 g. The latter was converted to the hydrochloride salt by dissolving in 325 ml. of hot water, filtering, and slowly adding 25 ml. of concentrated HCl to the warm mother liquor. The product was allowed to crystallize at room temperature overnight, then recovered by filtration with 5% aqueous HCl wash and dried *in vacuo* at 50°; yield 9.5 g. (57%); λ_{max}^{KBr} 5.72 μ; $\lambda_{max}^{MeOH-0.01\ N\ HCl}$ 237, 274, and 374 mμ; log ε 4.34, 4.27, and 3.60. The n.m.r. spectrum in trifluoroacetic acid shows signals at 3.97 and 4.38 τ. The product is not readily detected on paper chromatographs by ultraviolet fluorescence.

Anal. Calcd. for $C_{22}H_{21}N_2O_7Cl$ HCl: C, 53.1; H, 4.5; N, 5.6; Cl^-, 7.1. Found: C, 53.1; H, 4.5; N, 5.7; Cl^-, 6.7.

11a-Chloro-6-methylenetetracycline hydrochloride shows a bioassay value 5 to 10% that of 5-hydroxytetracycline. Treatment with excess $Na_2S_2O_4$ in 50% aq. methanol produces a bioassay equal to that of 5-hydroxytetracycline and a heavy 6-methylenetetracycline zone on paper chromatography.

There was no change in ultraviolet spectrum or bioassay when a sample of **7a** was refluxed for 30 min. in 2 *N* HCl, conditions which gave almost complete degradation of the starting hemiketal (**5a**) to 11a-chloroisotetracycline (**6a**).

11a-Fluoro-6-methylenetracycline (**7b**).—11a-Fluorotetracycline-6,12-hemiketal (250 mg.) was dissolved in 2 ml. of 60% perchloric acid and heated for 15 min. at 60–65°. Addition of water precipitated a crystalline product which was filtered off, washed with water, and dried. The yield of perchlorate salt of the product was 205 mg.; $\lambda_{max}^{MeOH-0.01\ N\ HCl}$ 236, 272, 365 mμ; λ_{max}^{KBr} 5.7, 6.0, 6.34, 9.17 μ (ClO_4^-). 6-Methylenetetracycline was identified by paper chromatography when this material (1 mg./ml. in 50% aqueous methanol) was reduced with excess sodium hydrosulfite.

11a-Chloro-6-methylene-5-hydroxytetracycline (**7c**).—11a-Chloro-5-hydroxytetracycline-6,12-hemiketal (5 g.) was dissolved in 15 ml. of liquid hydrogen fluoride employing an operating procedure similar to that for **7a**. After stirring for 3.5 hr. at 0–5° the reaction mixture was poured into 100 ml. of cold acetone containing 7.5 ml. of 47% aqueous hydriodic acid seeded with crystals of the product. After stirring in the ice bath for 45 min., the product was recovered by suction filtration with acetone wash and air-dried. The yield of crystalline hydriodide, containing 2 moles of acetone of crystallization, was 3.4 g. (47%). The product showed λ_{max}^{KBr} 5.70 (12-ketone) and 5.86 μ (acetone); $\lambda_{max}^{MeOH-0.01\ N\ HCl}$ 223, 271, and 374 mμ; log ε 4.47, 4.27, and 3.56.

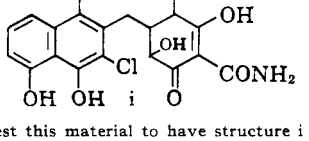
Anal. Calcd. for $C_{22}H_{21}N_2O_8Cl \cdot HI \cdot 2CH_3COCH_3$: C, 46.6; H, 4.8; N, 3.9; Cl, 4.9; I^-, 17.6; neut. equiv., 721. Found: C, 46.4; H, 4.8; N, 3.8; Cl, 5.0; I^-, 17.5; neut. equiv., 706.

The product may also be isolated from liquid HF as *p*-toluenesulfonate salt. For the acetone–HI mixture in the above preparation there is substituted 50 ml. of isopropyl alcohol, 50 ml. of acetone, and 5 g. of *p*-toluenesulfonic acid. The yield of acetone-washed, air-dried material is 5.6 g. (81%).

In early experiments the product was isolated as a crude hydrofluoride salt by adding the HF reaction mixture dropwise to 3 volumes of well-stirred ether. The product was isolated by filtration with thorough ether reslurries and washes. Contamination by lactone degradation product(s) was indicated by a weak infrared band near 5.6 μ (KBr pellet).

7,11a-Dichloro-6-methylene-5-hydroxytetracycline (**7d**).—11a-Chloro-5-hydroxytetracycline-6,12-hemiketal (**5c**, 10 g.) was dissolved in 30 ml. of liquid HF contained in a polyethylene bottle cooled in an ice–water bath. The solution was kept for 3 hr. in the bath to form the intermediate methylene compound **7c** *in situ*. N-Chlorosuccinimide (3.2 g.) was then added portion-

(35) An attempt to dry a sample *in vacuo* at 100° led to decomposition. Ultraviolet spectrum (1,8-dihydroxynaphthalene + tetracycline A-ring), infrared spectrum (no bands 5–6 μ), and insolubility in aq. sodium bicarbonate would suggest this material to have structure i which would result from lactone formation, 5a,6-elimination, and decarboxylation.



i

wise with stirring and the reaction kept for an additional hour in the bath. The reaction mixture was poured into a mixture of 200 ml. of isopropyl alcohol and 12 ml. of 57% aq. HI chilled to −15°. The temperature rose to 10°, and the hydriodide salt of the product crystallized heavily on stirring for a few minutes. The product was recovered by filtration with isopropyl alcohol and finally ether wash. The yield of 7,11a-dichloro-6-methylene-5-hydroxytetracycline hydriodide diisopropanolate was 7.4 g. (48%). A sample for analysis was obtained by recrystallization from hot isopropyl alcohol; λ_{max}^{KBr} 5.7 μ; $\lambda_{max}^{MeOH-0.01\ N\ HCl}$ 261 and 379 mμ, log ε 4.28 and 3.61.

Anal. Calcd. for $C_{22}H_{20}O_8N_2Cl_2 \cdot HI \cdot 2(CH_3)_2CHOH$: C, 44.3; H, 4.9; N, 3.7; Cl, 9.4; I, 16.7; iso-C_3H_7O, 15.6. Found: C, 44.3; H, 4.9; N, 3.6; Cl, 9.7; I, 16.4; iso-C_3H_7O, 15.5.

11a-Fluoro-6-methylene-5-hydroxytetracycline (7e).—Six grams of 11a-fluoro-5-hydroxytetracycline-6,12-hemiketal (**4b**) was dissolved in 45 ml. of hydrogen fluoride and allowed to stand at room temperature overnight. The solvent was then evaporated under a stream of nitrogen and the residue dissolved in methanol. This solution was poured into ether and the precipitate was filtered and dried; yield 5.1 g.; $\lambda_{max}^{MeOH-0.01\ N\ HCl}$ 236, 268, and 365 mμ; λ_{max}^{KBr} 5.7 μ. Hydrogenation of this material produced α- and β-6-deoxy-5-hydroxytetracyclines.[10c,11] Reduction of the same product (25 mg.) with 15 mg. of zinc powder in 0.625 ml. of water (15 min. stirring at room temperature) produced 6-methylene-5-hydroxytetracycline (at least 50% conversion, product identified by paper chromatographic and bioassay techniques).

6-Methylenetetracycline (10a).—A solution of 11a-chloro-6-methylenetetracycline hydrochloride (50 g.) in Methyl Cellosolve (500 ml.) was cooled to 4° in an ice–water bath and gradually treated with zinc dust (50 g.) at such a rate that the temperature did not rise above 10–12°. This required approximately 15 min. At the end of this time the excess zinc was removed by filtration, washed with Methyl Cellosolve, and treated gradually (over 5–10 min.) with 1 l. of water. A yellow solid separated rapidly. The slurry was cooled in an ice bath and adjusted to a pH of approximately 6.5 with 10% sodium hydroxide (38 ml.). This caused the separation of more solid. The heavy slurry of zinc complex salt was stirred in an ice bath for approximately an hour, filtered, and washed thoroughly with water. It was then removed from the funnel, repulped in water (750 ml.), then treated with excess concentrated hydrochloric acid (*ca.* 10 ml.). The zinc salt dissolved and was followed by crystallization of the hydrochloride salt. The slurry of hydrochloride was allowed to digest at room temperature for approximately 1 hr., filtered, and washed with 1% aqueous hydrochloric acid solution. The dry product weighed 37.8 g. (81% yield) and was essentially pure. For analysis, 0.5 g. of this material was dissolved in a minimum amount of methanol, filtered, then treated dropwise with concentrated hydrochloric acid with scratching until crystallization began. The slurry was left for several hours at room temperature, then filtered, and washed with methanol. The sample, dried at 60° *in vacuo* for 18 hr., showed apparent pK_a values of 3.5, 7.35, and 9.5 in 50% aq. ethanol; $\lambda_{max}^{MeOH-0.01\ N\ HCl}$ 254 and 353 mμ, log ε 4.35 and 4.19.

Anal. Calcd. for $C_{22}H_{22}N_2O_7 \cdot HCl$: C, 57.1; H, 5.0; N, 6.1; Cl^-, 7.7; neut. equiv., 463. Found: C, 57.1; H, 5.1; N, 6.1; Cl^-, 7.7; neut. equiv., 478.

6-Methylene-5-hydroxytetracycline (10b).—11a-Chloro-6-methylene-5-hydroxytetracycline (*p*-toluenesulfonate salt, 65 g.) was slurried in 1 l. of 50% methanol–water. Sodium hydrosulfite (17.4 g.) was added portionwise. After stirring for 15 min. at room temperature, the reaction mixture was treated with 10 g. of Darco KB and then filtered through a Supercel pad with 50% methanol–water wash. Upon treatment of the mother liquor with 50 g. of 5-sulfosalicylic acid, the product crystallized as the sulfosalicylate salt (63.8 g., 96%).

The sulfosalicylate salt (12.3 g.) was dissolved in a hot mixture of 60 ml. of acetone, 60 ml. of methanol, and 12 ml. of concentrated HCl. The solution was clarified, cooled, and stirred overnight. A 62% yield (5.5 g.) of solvated hydrochloride[36] resulted; $\lambda_{max}^{MeOH-0.01\ N\ HCl}$ 253 and 345 mμ, log ε 4.37 and 4.19.

Anal. Calcd. for $C_{22}H_{22}O_8N_2 \cdot HCl \cdot 0.5H_2O \cdot 0.5CH_3OH$: C, 53.6; H, 5.2; N, 5.6; Cl^-, 7.0; H_2O, 1.8; OCH_3, 3.0. Found: C, 54.0; H, 5.3; N, 5.4; Cl^-, 6.9; H_2O, 1.9; OCH_3, 3.1.

The 6-methylene-5-hydroxy compound (**10b**) was also prepared from 5-hydroxytetracycline sulfuric acid ester (**16**). The ester (1 g.) was dissolved in 15 ml. of liquid HF maintained in an ice–water bath. The solution was stirred for 1 hr. at 0–5°. The HF was evaporated under a stream of nitrogen and the residue stirred for 1 hr. with 30 ml. of ether. The crude product, 0.97 g., was recovered by filtration. Paper chromatography showed 6-methylene-5-hydroxytetracycline and unreacted ester to be among the compounds present in the crude. The former was separated by dissolving 50 mg. of crude in 0.7 ml. of water and 0.7 ml. of methanol containing 50 mg. of 5-sulfosalicylic acid, followed by immediate filtration. The sulfosalicylate salt of 6-methylenetetracycline (8.4 mg., 14% yield) crystallized from the mother liquor on standing overnight.

A nonsolvated form of the hydrochloride[36] was obtained by dissolving 10 g. of solvated hydrochloride in 300 ml. of water. The former crystallized on standing overnight; yield 6.3 g. (67%).

Anal. Calcd. for $C_{22}H_{22}O_8N_2 \cdot HCl$: C, 55.2; H, 4.8; N, 5.9; Cl, 7.4. Found: C, 55.1; H, 4.9; N, 5.9; Cl, 7.4.

Amphoteric 6-methylene-5-hydroxytetracycline was obtained by dissolving 5 g. of the nonsolvated hydrochloride in 150 ml. of methanol and 4 ml. of water containing 1.42 ml. of triethylamine. The solution, obtained by brief stirring, was filtered immediately. The product, 3.9 g. (84%), crystallized on standing overnight. For analysis, a sample was dried at 100° *in vacuo*.

Anal. Calcd. for $C_{22}H_{22}N_2O_8$: C, 59.7; H, 5.0; N, 6.3. Found: C, 59.7; H, 5.0; N, 6.4.

6-Methylene-5-hydroxytetracycline Methiodide.—Amphoteric 6-methylene-5-hydroxytetracycline (10 g.) was added to a solution of 25 ml. of methyl iodide in 125 ml. of tetrahydrofuran. The mixture was refluxed for 5 hr. After 2 hr., complete solution occurred. After 3 hr., the methiodide began to crystallize. The reaction mixture was cooled to room temperature and the product recovered by filtration with tetrahydrofuran wash; yield 11.8 g. (89%). Paper chromatography and bioassay indicated *ca.* 1% contamination by starting material. For analysis, a sample was recrystallized from hot methanol and dried *in vacuo* at 50°; $\lambda_{max}^{MeOH-0.01\ N\ HCl}$ 240 and 347 mμ, log ε 4.33 and 4.17.

Anal. Calcd. for $C_{23}H_{25}N_2O_8I$: C, 47.3; H, 4.4; N, 4.8; I^-, 21.7. Found: C, 47.2; H, 4.4; N, 4.2; I^-, 21.1.

4-Dedimethylamino-6-methylene-5-hydroxytetracycline.—A well-stirred solution of 5.8 g. of 6-methylene-5-hydroxytetracycline methiodide in 9.7 ml. of acetic acid and 9.7 ml. of water was treated with 5.8 g. of zinc dust. The temperature was maintained at 24–27° by means of a 20° water bath. After 15 min., excess zinc was removed by filtration with limited acetic acid wash. The mother liquor was added slowly to 580 ml. of ice and water containing 5.6 ml. of concentrated hydrochloric acid. The precipitated product, 2.8 g. (65%), was recovered by filtration with water wash. The product was recrystallized by dissolving 1 g. in 10 ml. of boiling methanol. It crystallized on standing, was recovered by filtration, and was dried at 60° *in vacuo;* yield 0.8 g.; $\lambda_{max}^{MeOH-0.01\ N\ HCl}$ 242 and 351 mμ, log ε 4.36 and 4.19.

Anal. Calcd. for $C_{20}H_{17}NO_8 \cdot CH_3OH$: C, 58.5; H, 4.9; N, 3.2; CH_3O, 7.2. Found: C, 58.4; H, 4.9; N, 3.1; CH_3O, 6.4.

7-Chloro-6-methylene-5-hydroxytetracycline (10c).—7,11a-Dichloro-6-methylene-5-hydroxytetracycline hydroiodide diisopropanolate (9.4 g.) was stirred with 60 ml. of methanol and 60 ml. of water. Sodium hydrosulfite (4.3 g.) was added and the mixture stirred for 1 hr. at room temperature. The pH was adjusted to 1.2 with hydrochloric acid and insolubles removed by filtration. 5-Sulfosalicylic acid dihydrate (2 g.), dissolved in 10 ml. of methanol, was added to the filtrate. The 5-sulfosalicylate salt of the product crystallized on standing (4.9 g., 57%). The sulfosalicylate salt was slurried in 20 ml. of methanol and the apparent pH adjusted to 8.5 with triethylamine. The solution was charcoaled, filtered, and the pH readjusted to 5.5 with hydrochloric acid. The amphoteric material crystallized and was recovered by filtration with thorough water and then methanol washes; yield of air-dried product, 2.5 g. (73%).

The base was reconverted to analytical grade sulfosalicylate by dissolving 1 g. in a solution of 1 g. of 5-sulfosalicylic acid dihydrate in 13 ml. of methanol, followed by immediate filtration. 7-Chloro-6-methylene-5-hydroxytetracycline 5-sulfosalicylate monohydrate, which crystallized on standing, was recovered by filtration and air-dried. The yield was 1.2 g. (80%); $\lambda_{max}^{MeOH-0.01\ N\ HCl}$ 239, 268, 346 mμ; log ε 4.46, 4.07, and 4.11; $\lambda_{inf}^{MeOH-0.01\ N\ HCl}$ 258 and 366 mμ, log ε 4.33 and 4.08.

Anal. Calcd. for $C_{22}H_{21}O_8N_2Cl \cdot C_7H_6O_6S \cdot H_2O$: C, 48.8; H, 4.1; N, 3.9; Cl, 5.0; S, 4.5; volatile, 2.5. Found: C, 48.9; H, 4.0; N, 4.0; Cl, 5.1; S, 4.4; volatile, 2.3.

The amphoteric material was also converted to hydrochloride; 1 g. was slurried in 7.5 ml. of isopropyl alcohol. Dry HCl was bubbled into the slurry until solution occurred (*ca.* 20 min.; increase in weight was 0.5 g.). External cooling was employed during this process so that the temperature did not rise above 35°. The solution was filtered and the filtrate refluxed on a steam bath for 45 min. The product crystallized heavily and, after cooling to room temperature, was recovered by filtration. The yield of 7-chloro-6-methylene-5-hydroxytetracycline hydrochloride, dried *in vacuo* for 48 hr. at 50°, was 0.86 g. (80%). It showed

(36) In later pilot plant scale experiments by Messrs. H. E. Klei, Jr., and R. M. Gifford, nonsolvated hydrochloride crystallized in higher yield when a very similar crystallization method was employed. Subsequently, we were able to obtain only the nonsolvated form by this method. Some modification in some of the mercaptan addition reactions may be necessary to accommodate the nonsolvated hydrochloride, which is, in general, less soluble.

$\lambda_{max}^{MeOH-0.01N\ HCl}$ 445 and 345 mμ, log ε 4.36 and 4.11; $\lambda_{inf}^{MeOH-0.01\ N\ HCl}$ 366 mμ, log ε 4.07. The presence of only two aromatic hydrogens is indicated by two doublets at τ 2.4 and 3.1 in the n.m.r. (F_3CCOOH).

Anal. Calcd. for $C_{22}H_{21}O_8N_2Cl\cdot HCl$: C, 51.5; H, 4.3; N, 5.5; Cl, 13.8: Cl^-, 6.9. Found: C, 51.7; H, 4.4; N, 5.4; Cl, 13.7; Cl^-, 6.9.

Exhaustive Methylation–Oxidation of 7-Chloro-6-methylene-5-hydroxytetracycline.—A solution of 1.5 g. of 7-chloro-6-methylene-5-hydroxytetracycline in 60 ml. of H_2O was adjusted to pH 10.5 with 50% NaOH. The solution was stirred and 15 ml. of dimethyl sulfate was added dropwise over 30 min., keeping the pH of the reaction higher than 10 with additional 50% NaOH. The reaction was heated on the steam bath for 1 hr. and then cooled to 25° and treated with 10 g. of $KMnO_4$ dissolved in 60 ml. of H_2O. After stirring at 25° for 1 hr., 5 g. of $KMnO_4$ was added and the suspension was stirred and heated on a steam bath for 16 hr. The suspension was cooled to 25°, acidified with concentrated H_2SO_4, and treated with sufficient Na_2SO_3 to dissolve all of the MnO_2. The resulting solution was continuously extracted with ether for 2 days. The residue obtained after drying and evaporation of the ether was sublimed at 90° (10^{-4} mm.) to yield 104 mg. of material that was slurried in 5 ml. of ether and filtered to provide 61 mg. of 6-chloro-3-methoxyphthalic anhydride, m.p. 185–186° dec., lit.[26] m.p. 187–188°. The infrared and ultraviolet spectra of the material were identical with a sample obtained by a similar methylation–oxidation[26] sequence from 7-chlorotetracycline.

5a,6-Anhydrotetracycline (11a).—Methylenetetracycline hydrochloride (1.0 g.) was dissolved under nitrogen in 5 ml. of concentrated H_2SO_4. After standing for 1 min., the reaction was quenched with 20 ml. of water and the pH adjusted to 4.5. The aqueous solution was extracted with ether. The ether was dried over anhydrous Na_2SO_4 and then stripped to dryness to yield 100 mg. of crystalline 5a,6-anhydrotetracycline, identified by paper chromatography and spectra. Tetracycline treated in a like manner gave a similar yield of 5a,6-anhydrotetracycline.

Apoterramycin (12).—*6-Methylene-5-hydroxytetracycline* (1 g.) was slurried in 15 ml. of acetone and 5 ml. of concentrated HCl. The mixture was refluxed *ca.* 18 hr. After cooling to room temperature, the mixture was filtered and 20 mg. of insoluble material discarded. The mother liquor was adjusted to pH 6 with aq. NaOH. Amorphous α- and β-apoterramycins (230 mg., identified by spectra and paper chromatography) precipitated. Crystalline α-apoterramycin, identical with that obtained earlier from 5-hydroxytetracycline,[5a] was crystallized from ethanol.[5a]

Lactone Degradation Product (13) from 11a-Chloro-6-methylene-5-hydroxytetracycline.—A high degree of degradation to lactone was first noted when an attempt was made to convert crude 11a-chloro-6-methylene-5-hydroxytetracycline hydrofluoride to the amphoteric form. An aqueous solution of the salt, adjusted to pH 4.5, gave high recovery of a precipitate having much more lactone absorption and much less 12-ketone absorption than the starting material. Nearly complete conversion to lactone was obtained by dissolving 8 g. of 11a-chloro-6-methylene-5-hydroxytetracycline in 50 ml. of water and adjusting the pH to *ca.* 5.5 with saturated aqueous sodium bicarbonate. The slurry obtained was stirred overnight under nitrogen and the crude product (6.1 g.) isolated by filtration with water wash. The crude was dissolved in 1500 ml. of acetone, charcoaled, filtered, and the mother liquor stripped to dryness to yield 4.4 g. of crystalline lactone degradation product showing clean λ_{max}^{KBr} 5.6 μ and no λ_{max}^{KBr} 5.7 μ.

An analytical sample was prepared by dissolving 500 mg. in 10 ml. of methanol. Concentrated hydrochloric acid (0.5 ml.) was added. The hydrochloride crystallized on standing at room temperature. The yield of product, dried *in vacuo*, was 134 mg.; It showed λ_{max}^{KBr} 5.6μ; $\lambda_{max}^{MeOH-0.01\ N\ HCl}$ 239 μ, 268, and 362 mμ; log ε 4.38, 4.33, and 3.68.

Anal. Calcd. for $C_{22}H_{21}O_8N_2Cl\cdot 0.5H_2O\cdot HCl$: C, 50.6; H, 4.4; N, 5.4; Cl, 13.6. Found: C, 50.7; H, 4.6; N, 5.5; Cl, 13.7.

Ozonolysis Studies.—11a-Chloro-6-methylenetetracycline hydrochloride (1.5 g.) was dissolved in 150 ml. of water. 1-Octanol (1 ml.) was added to prevent excessive foaming. The solution was ozonized for 30 min. at 0°, using 1.14 cu. ft. of ozonized oxygen containing 6.5 vol. % of ozone. The solution was then hydrogenated for 4 hr. in the presence of 200 mg. of 10% palladium-on-charcoal catalyst (hydrogen uptake, 65.6 ml.). Steam was passed into the hydrogenation mixture and about 100 ml. of distillate collected. To this a solution of 500 mg. of dimedone in 20 ml. of ethanol was added and the mixture refluxed for 15 min. The dimedone–formaldehyde adduct crystallized on cooling. It was filtered off, washed with water, and dried; yield 170 mg. (19%), m.p. 192°, no depression of m.p. on admixture of authentic adduct.

6-Methylenetetracycline hydrochloride (1.5 g.) was ozonized and hydrogenated as described above (1.5 cu. ft. of O_3/O_2, 36 min.; 68 ml. of H_2, 4 hr.). The yield of dimedone–formaldehyde adduct was 220 mg. (22.5%), m.p. 192°.

11a-Chloro-6-methylene-5-hydroxytetracycline hydrochloride (1.5 g.) was ozonized in 150 ml. of water and 1 ml. of 1-octanol at 0° for 1 hr. and at 30° for 1 hr., using 3.5 cu. ft. of ozonized oxygen. The solution was hydrogenated in the presence of 500 mg. of 5% palladium-on-charcoal (50% wet). The hydrogen uptake was 109 ml. The catalyst was filtered off and the filtrate refluxed for 15 min. with a solution of 600 mg. of dimedone in 20 ml. of ethanol. From the cooled solution 230 mg. (25.6%) of dimedone–formaldehyde adduct, m.p. 192°, was isolated.

Parallel experiments on 5-hydroxytetracycline and on 11a-chlorotetracycline-6,12-hemiketal gave no significant amount of dimedone–formaldehyde adduct.

5-Hydroxytetracycline Sulfuric Acid Ester (14).—To a stirred solution of 4.6 g. (0.01 mole) of anhydrous oxytetracycline in 40 ml. of dry tetrahydrofuran was added 3.5 g. (0.022 mole) of pyridine–sulfur trioxide complex. The resulting suspension was stirred at 25° for 16 hr. and filtered. The precipitate was stirred in 25 ml. of 2% HCl for 30 min., filtered, and washed with methanol and then ether to provide 4.2 g. (78%) of pure 5-hydroxytetracycline sulfuric acid ester, $\lambda_{max}^{MeOH-0.01\ N\ HCl}$ 262 and 332 mμ, log ε 4.39 and 3.76.

Anal. Calcd. for $C_{22}H_{24}N_2O_{12}S$: C, 48.89; H, 4.48; N, 5.18; S, 5.93. Found: C, 48.60; H, 4.52; N, 4.89; S, 5.64.

A suspension of 2 g. of 5-hydroxytetracycline sulfuric acid ester in 50 ml. of methanol was refluxed overnight. An 80% reconversion of 5-hydroxytetracycline resulted (paper chromatographic, ultraviolet, and bioassay evidence).

13-Phenylmercapto-α-6-deoxytetracycline (19a).—6-Methylenetetracycline hydrochloride (5 g.) was dissolved in 50 ml. of 1-butanol, 25 ml. of ethylene glycol, and 50 ml. of phenyl mercaptan. The solution was heated on a steam bath for 1.2 hr. The reaction mixture was cooled to room temperature and 250 ml. of 1,2-dimethoxyethane added. Crystalline hydrochloride of the product (4.6 g., 81%) was obtained by adding this solution slowly to 2 l. of stirring ether. An analytical sample was prepared by dissolving the hydrochloride (500 mg.) in 4 ml. of methanol. Following clarification, *p*-toluenesulfonic acid (400 mg.) was added and dissolved with stirring. 13-Phenylmercapto-α-6-deoxytetracycline *p*-toluenesulfonate crystallized on standing. It was dried *in vacuo* at 85° for analysis; $\lambda_{max}^{MeOH-0.01\ N\ HCl}$ 256 and 356 mμ, log ε 4.41 and 4.13.

Anal. Calcd. for $C_{28}H_{28}N_2O_7S\cdot C_7H_8O_3S$: C, 59.3; H, 5.1; N, 3.9; S, 9.0. Found: C, 58.8; H, 5.2; N, 3.7; S, 9.4.

When the above reaction was carried out in the presence of 500 mg. of 2,2′-azobis-(2-methylpropionitrile), complete conversion was accomplished in < 10 min. Hydrogen peroxide (*ca.* 0.5 ml. of 30% aqueous) was also effective as catalyst, although contamination of the product by the corresponding sulfoxide was noted.

13-Phenylmercapto-α-6-deoxy-5-hydroxytetracycline (19b).—A mixture of 250 ml. of 1-butanol, 125 ml. of ethylene glycol, 25 g. of 6-methylene-5-hydroxytetracycline hydrochloride (solvated),[36] and 250 ml. of phenyl mercaptan under nitrogen was stirred with heating to obtain a solution. 2,2′-Azobis-(2-methylpropionitrile) (2.5 g.) was added and the reaction stirred for 2 hr., on the steam bath under nitrogen. The mixture was then steam distilled until there was essentially one phase in the distillate. The pH of the aqueous residue was adjusted to 4.5 and the amorphous, amphoteric product recovered by filtration with water wash. The yield of air-dried 13-phenylmercapto-α-6-deoxy-5-hydroxytetracycline was 22.4 g. (90%). For analyses, a sample was recrystallized from hot benzene; $\lambda_{max}^{MeOH-0.01\ N\ HCl}$ 259 and 347 mμ, log ε 4.41 and 4.07.

Anal. Calcd. for $C_{28}H_{28}N_2O_8S$: C, 60.9; H, 5.1; N, 5.1; S, 5.8. Found: C, 61.0; H, 5.1, N, 4.7; S, 5.8.

13-Benzylmercapto-α-6-deoxytetracycline (19c).—A mixture of 50 g. of 6-methylenetetracycline hydrochloride and 5 g. of 2,2′-azo-bis-(2-methylpropionitrile) in 500 ml. of benzyl mercaptan, 500 ml. of 1-butanol, and 250 ml. of ethylene glycol was stirred for 30 min. in a steam bath. The reaction mixture was steam distilled until the distillate was essentially one phase. The pot material, which had been maintained at *ca.* 1-l. volume, was cooled to room temperature and filtered. The product was precipitated from the filtrate, chilled in an ice–water bath, by the addition of 500 ml. of concentrated hydrochloric acid. After standing 0.5 hr., the mixture was filtered by suction, with 5% aq. HCl wash. The wet cake was taken up into 500 ml. of hot methanol containing 50 g. of *p*-toluenesulfonic acid. The volume was reduced to 300 ml. and the seeded solution allowed to crystallize by standing overnight at room temperature. The product was recovered by filtration. It was washed with acetone, repulped with 150 ml. of acetone, and finally refiltered. The yield of crystalline *p*-toluenesulfonate salt of the product was 37.1 g. (48%). For analyses, 1 g. of this material was dissolved in 25 ml. of acetone by the addition of 0.7 ml. of concentrated H_2SO_4. The solution was filtered. Crystallization of the *p*-toluenesulfonate salt was caused by the addition of 70 ml. of water. After

digesting for 1.5 hr., the purified product (600 mg.) was recovered by filtration with acetone and finally ether wash. It was dried *in vacuo* for 48 hr. at room temperature; $\lambda_{max}^{MeOH-0.01\ N\ HCl}$ 267 and 358 mμ, log ε 4.27 and 4.14.

Anal. Calcd. for $C_{29}H_{30}N_2O_7S \cdot C_7H_8O_3S$: C, 59.8; H, 5.3; N, 3.9; S, 8.8; C-methyl, 0.00. Found: C, 59.8; H, 5.5; N, 3.5; S, 8.9; C-methyl, 0.5.

13-Benzylmercapto-α-6-deoxy-5-hydroxytetracycline (19d).—6-Methylene-5-hydroxytetracycline hydrochloride (40 g., solvated form[36]), was dissolved at room temperature in 400 ml. of 1-butanol, 200 ml. of ethylene glycol, and 400 ml. of benzyl mercaptan by stirring at room temperature. There was then added 4 g. of 2,2′-azo-bis-(2-methylpropionitrile) and the mixture was stirred for 2 hr. on a steam bath under nitrogen. The reaction mixture was then steam distilled until there was very little organic phase in the distillate. The pot residue (*ca.* 1 l.) was cooled to room temperature and filtered. The filtrate was adjusted to pH 4.5 with aq. NaOH and the product recovered by filtration with water wash. The yield of crude, dried *in vacuo* over KOH, was 40.4 g. Ten grams of crude product was crystallized by dissolving in 2 l. of hot methylene chloride (0.7 g. of insolubles), treating with 5 g. Darco G-60, boiling down to 600 ml., and adding 400 ml. of benzene. Crops of material were then obtained by gradual reduction of the volume by boiling followed by cooling to room temperature. Early crops (1.8 g., down to a volume of 500 ml.) contained decreasing amorphous material and decreasing contamination by a material showing an infrared band at 5.8 μ (KBr pellet). Intermediate crops (5.2 g.) down to a volume of 90 ml. were crystalline and free of the earlier or later contaminants detected by infrared. A final crop (0.4 g.) down to a volume of 20 ml. showed a shoulder near 5.6 μ. The final residue (0.8 g.) showed very little of the desired product on paper chromatography. It exhibited a band at 5.63 μ (KBr pellet).

An intermediate crop, dried *in vacuo* at 50°, showed $\lambda_{max}^{MeOH-0.01\ N\ HCl}$ 266 and 348 mμ, log ε 4.31 and 4.06.

Anal. Calcd. for $C_{29}H_{30}N_2O_8S$: C, 61.5; H, 5.3; N, 4.9; S, 5.7. Found: C, 61.7; H, 5.4; N, 4.7; S, 6.0.

13-(2-Hydroxyethylmercapto)-α-6-deoxy-5-hydroxytetracycline (19e).—6-Methylene-5-hydroxytetracycline hydrochloride (20 g., solvated form),[36] 200 ml. of 2-hydroxyethyl mercaptan, and 4 ml. of 30% aq. hydrogen peroxide were heated on a steam bath for 35 min. The solution was cooled to room temperature and added slowly to 2 l. of well-stirred ether. The precipitated crude product (23 g.) was recovered by filtration. Crude product (12.5 g.) was partitioned in a large column employing 2 kg. of cellulose powder with water as stationary phase and ethyl acetate as mobile phase. A center cut of fractions gave, on stripping to dryness, 5.0 g. of material free of polar and nonpolar impurities. Columned material (400 mg.) was dissolved in 4 ml. of water and the solution clarified. *p*-Toluenesulfonic acid (300 mg.) was added. Sufficient 3A ethanol (2.0 ml.) was added to dissolve the precipitate and the solution immediately filtered. The *p*-toluenesulfonate salt of 13-(2-hydroxyethylmercapto)-α-6-deoxy-5-hydroxytetracycline crystallized on standing. The yield of material, dried *in vacuo* at room temperature, was 228 mg. (19% over-all). The ultraviolet spectrum showed $\lambda_{max}^{MeOH-0.01\ N\ HCl}$ 264 and 347 mμ, log ε 4.31 and 4.06.

Anal. Calcd. for $C_{24}H_{28}N_2O_9S \cdot C_7H_8O_3S \cdot H_2O$: C, 52.4; H, 5.4; N, 3.9; O, 29.3; S, 9.0: H_2O, 2.5. Found: C, 52.5; H, 5.2; N, 3.8; O, 29.4; S, 9.0; H_2O, 2.3.

13-Acetylmercapto-α-6-deoxytetracycline (19f).—6-Methylenetetracycline hydrochloride (10 g.) and 1 g. of 2,2′-azobis-(2-methylpropionitrile) were dissolved in 100 ml. of thiolacetic acid at room temperature. The solution was heated for 2 hr. at 70°. An additional gram of catalyst was added and heating continued for 2 more hours. The cooled reaction mixture was poured slowly into 500 ml. of rapidly stirring ether. The crude product (11.7 g.) was recovered by filtration and air-dried. Crude product (3.0 g.) was heated in 30 ml. of methanol and filtered. *p*-Toluenesulfonic acid (6 g.) in 10 ml. of methanol was added to the mother liquor. Crystalline 13-acetylthio-α-6-deoxytetracycline *p*-toluenesulfonate was recovered by filtration. The yield of product dried *in vacuo* was 1.2 g. (32% over-all); λ_{max}^{KBr} 5.93 μ and $\lambda_{max}^{MeOH-0.01\ N\ HCl}$ 268 and 355 mμ, log ε 4.30 and 4.16.

Anal. Calcd. for $C_{24}H_{26}N_2O_8S \cdot C_7H_8O_3S$: C, 55.2; H, 5.1; N, 4.2; S, 9.5. Found: C, 55.0; H, 5.0; N, 4.0; S, 9.3.

13-Acetylmercapto-α-6-deoxy-5-hydroxytetracycline (19g).—6-Methylene-5-hydroxytetracycline hydrochloride (solvated form, 5 g.),[36] 500 mg. of 2,2′-azobis-(2-methylpropionitrile), and 50 ml. of thiolacetic acid were stirred under nitrogen and refluxed for 2 hr. The mixture was cooled to room temperature, another 500 mg. of the azo catalyst added, and the mixture refluxed for an additional 2 hr. The warm reaction mixture was added slowly to 500 ml. of stirring ether. The crude product (5.4 g.) was recovered by filtration with ether wash. Crude product (2.3 g.) was dissolved in 15 ml. of methanol and clarified by filtration. Water (20 ml.) and 5-sulfosalicylic acid dihydrate (2.3 g.) was added to the filtrate. An immediate amorphous precipitate was removed by filtration and discarded. On standing in the refrigerator, 13-acetylmercapto-α-6-deoxy-5-hydroxytetracycline crystallized as the 5-sulfosalicylate salt. The yield of product, dried *in vacuo*, was 1.0 g. (30% over-all). It showed λ_{max}^{KBr} 5.95 μ (composite of acetyl, thiocarbonyl, and the carboxyl group of 5-sulfosalicyclic acid—considerably more intense than carbonyl bands near 6.2 and 6.3 μ; $\lambda_{max}^{MeOH-0.01\ N\ HCl}$ 268, 317 and 348 mμ; log ε 4.30, 4.06, and 4.11.

Anal. Calcd. for $C_{24}H_{26}N_2O_9S \cdot C_7H_6O_6S$: C, 50.6; H, 4.4; N, 3.8; S, 8.7. Found: C, 51.0; H, 4.5; N, 3.5; S, 8.6.

13-Phenylmercapto-α-6-deoxytetracycline S-Oxide (22a).—13-Phenylmercapto-α-6-deoxytetracycline hydrochloride (1 g.) was dissolved in 28 ml. of methanol by stirring at room temperature. Aqueous hydrogen peroxide (0.30 ml. of 30%) was added and the solution left to stand at room temperature for 16 hr. A negative starch–iodide test was noted. The solution was air evaporated to dryness. Ether was added to the glassy residue, the solid was broken up, and the crude product recovered by filtration (0.99 g.). The crude (500 mg.) was dissolved in a hot mixture of 15 ml. of methanol and 10 ml. of acetone and filtered. *p*-Toluenesulfonic acid (350 mg.) was added and the solution boiled down to *ca.* 8 ml. The *p*-toluenesulfonate salt crystallized on standing. The yield of product, dried at 80° *in vacuo*, was 274 mg. (40% over-all); $\lambda_{max}^{MeOH-0.01\ N\ HCl}$ 257 and 355 mμ, log ε 4.32 and 4.17.

Anal. Calcd. for $C_{28}H_{28}N_2O_8S \cdot C_7H_8O_3S$: C, 58.0; H, 5.0; N, 3.9; S, 8.8. Found: C, 58.5; H, 4.9; N, 3.7; S, 8.7.

13-Benzylmercapto-α-6-deoxytetracycline S-Oxide (22b).—13-Benzylmercapto-α-6-deoxytetracycline *p*-toluenesulfonate (5 g.) was dissolved with stirring at room temperature in 500 ml. of methanol. Aqueous hydrogen peroxide (1.5 ml. of 30%) was added with stirring. The solution was permitted to stand overnight at room temperature. A small amount of insoluble material was removed by filtration. The filtrate was evaporated to dryness. The residue was stirred with excess ether and the crude product (5.2 g.) recovered by filtration. Crude product (600 mg.) was dissolved by stirring at room temperature in 6 ml. of acetone and 1 ml. of 2B ethanol. Insolubles were removed by filtration with 3 ml. of acetone wash. The *p*-toluenesulfonate salt of the product crystallized on standing overnight. The yield of product dried *in vacuo* was 170 mg. (29% over-all); $\lambda_{max}^{MeOH-0.01\ N\ HCl}$ 267 and 352 mμ, log ε 4.28 and 4.17.

Anal. Calcd. for $C_{29}H_{30}N_2O_8S \cdot C_7H_8O_3S$: C, 58.5; H, 5.2; N, 3.8; S, 8.9. Found: C, 58.8; H, 5.1; N, 3.5; S, 8.4.

13-Mercapto-α-6-deoxy-5-hydroxytetracycline (23).—13-Acetylmercapto-α-6-deoxy-5-hydroxytetracycline sulfosalicylate (2 g.) was dissolved by shaking in 100 ml. of concentrated HCl. The solution was allowed to stand for 5 days at room temperature. The mixture was poured into 800 ml. of ice and water, permitted to warm to room temperature, and then extracted with butanol. The butanol extracts were stripped to dryness, the residue slurried with ether, and the product (1.4 g., 69%) recovered by filtration. No starting material was detected by paper chromatography.

An analytical sample was prepared by recrystallization from hot methanol. It showed λ_{max}^{KBr} 5.95 μ (weaker than carbonyl bands near 6.2 and 6.3 μ, consistent with the carboxyl of sulfosalicylic acid, but no acetylmercapto group); $\lambda_{max}^{MeOH-0.01\ N\ HCl}$ 267, 317, and 350 mμ; log ε 4.29, 4.03, and 4.11; and a positive color reaction with sodium nitroprusside.

Anal. Calcd. for $C_{22}H_{24}N_2O_8S \cdot C_7H_6O_6S \cdot 2H_2O$: C, 47.6; H, 4.7; N, 3.8; S, 8.8; H_2O, 4.9. Found: C, 47.3; H, 5.0; N, 3.7; S, 8.7; H_2O, 3.8.

11a,13-Epithio-α-6-deoxytetracycline (24) and 7,13-Epithio-α-6-deoxytetracycline (25).—13-Benzylmercapto-α-6-deoxytetracycline S-oxide *p*-toluenesulfonate (5 g.) was dissolved with stirring in 150 ml. of concentrated HCl. The solution was allowed to stand for 7 days at room temperature. A small amount of insoluble material was removed by filtration. The mother liquor was diluted with 500 ml. of ice and water and then extracted with seven 100-ml. portions of 1-butanol. The combined butanol extracts were charcoaled, filtered, and stripped to dryness on a rotating evaporator. Ether was added to the residue and the crude product (3.0 g.) recovered by filtration. The crude (1.0 g.) was dissolved in 20 ml. of methanol and filtered. Concentrated HCl (0.5 ml.) was added to the mother liquor. On standing at room temperature, white crystals of 11a,13-epithio-α-6-deoxytetracycline hydrochloride were deposited. The yield of product, dried *in vacuo* at room temperature, was 265 mg. (24% over-all); λ_{max}^{KBr} 5.75 μ, $\lambda_{max}^{MeOH-0.01\ N\ HCl}$ 262 and 346 mμ, log ε 4.30 and 3.70.

Anal. Calcd. for $C_{22}H_{22}N_2O_7S \cdot HCl$: C, 53.4; H, 4.7; N, 5.7; S, 6.5; Cl^-, 7.2. Found: C, 53.1; H, 4.5; N, 5.2; S, 6.6; Cl^-, 6.9.

The mother liquor from the 11a,13-epithio isolation described above was evaporated down to 8 ml. and refrigerated overnight. A mixture of the two epithio compounds (149 mg.) crystallized.

The mother liquor was evaporated in air to *ca.* 2 ml. Yellow crystals (100 mg., 9% over-all) of 7,13-epithio-α-6-deoxytetracycline were obtained. An analytical sample was prepared by recrystallization from hot methanol, with acetone wash. It showed λ_{max}^{KBr}, no bands between 5 and 6 μ: $\lambda_{max}^{MeOH-0.01\ N\ HCl}$ 251, 337, and 420 mμ, log ε 4.38, 4.08, and 3.64; $\lambda_{max}^{MeOH-0.01\ N\ NaOH}$ 248 and 392 mμ, log ε 4.18 and 4.40.

Anal. Calcd. for $C_{22}H_{22}N_2O_7S \cdot HCl \cdot H_2O$: C, 51.5; H, 4.9; N, 5.5; S, 6.3; Cl^-, 6.9; H_2O, 3.5. Found: C, 51.4; H, 4.9; N, 5.2; S, 6.2; Cl^-, 7.1; H_2O, 3.4.

11a-Bromo-4-dedimethylaminotetracycline, Its 6,12-Hemiketal, and 9-Bromo-5a,6-anhydro-4-dedimethylaminotetracycline.—Following the procedure of Green, Wilkinson, and Boothe[16] for the preparation of 11a-bromo-4-dedimethylaminotetracycline in acetic acid on a 10-g. scale, a slight excess of bromine (1.37 ml., 1.07 equiv., rather than 1.28 ml., 1.0 equiv.) was inadvertently employed. There resulted, apparently because of a slight imbalance in acidity, a direct yield of 11.6 g. (100%) of crystalline 9-bromo-5a,6-anhydro-4-dedimethylaminotetracycline.[16] On a second 10-g. scale preparation the bromine was reduced to 1.25 ml. (0.98 equiv.) and the sodium acetate increased to 2.25 g. (rather than 2.05 g.). In this case 7.1 g. (60%) of pure 11a-bromo-4-dedimethylaminotetracycline crystallized directly from the reaction mixture—thus avoiding precipitation and crystallization steps. The product showed a strong infrared band at 5.77μ in KBr and in dioxane. Recrystallization of this material from chloroform gave 11a-bromo-4-dedimethylaminotetracycline-6,12-hemiketal (λ_{max}^{KBr} no bands 5 to 6 μ, identical with that of the material prepared by the N-bromosuccinimide–chloroform method of Green, Wilkinson, and Boothe[16]; $\lambda_{max}^{dioxane}$ 5.77μ, identical with that of material prepared by either method).

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Mechanisms of Photochemical Reactions in Solution. XIX. Photodimerization of Methyl β-Naphthyl Ether

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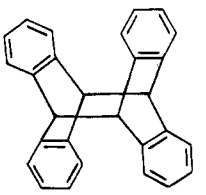
Methyl β-naphthyl ether dimerizes upon irradiation with ultraviolet light in a variety of solvents. Other naphthalenes, monosubstituted in the α- or β-position by methyl, hydroxy, bromo, amino, and in the α-position by methoxy groups, do not show this phenomenon. The photodimer is probably formed by a 1,4–1,4 dimerization of the unsubstituted ring through an excited singlet state. Benzophenone efficiently quenches the reaction, probably by accepting singlet excitation from the naphthyl ether in an energy transfer process.

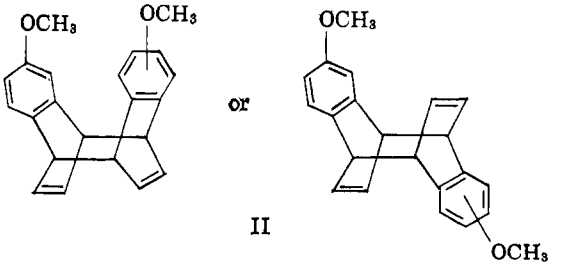
Unlike anthracenes,[2] naphthalene and its derivatives have not hitherto been reported to undergo photodimerization. It has now been found in these laboratories that methyl β-naphthyl ether dimerizes in a variety of solvents upon irradiation with ultraviolet light of wave lengths shorter than 3300 Å. The photodimer was almost completely insoluble in all solvents except strong acids. This insolubility probably aids in its formation. On being heated to its decomposition point (155–160°) or dissolution in strong acid, the photodimer reverts to methyl β-naphthyl ether. Related compounds such as methyl α-naphthyl ether, α- and β-methylnaphthalenes, α- and β-bromonaphthalenes, α- and β-naphthylamines, α- and β-naphthols, and naphthalene itself did not undergo dimerization under the same conditions. No product was formed on irradiation of methyl β-naphthyl ether in the presence of either maleic anhydride or dimethyl acetylenedicarboxylate.

The ultraviolet absorption spectrum of the photodimer offers very convincing evidence as to its structure. The positions and intensities for maxima in the spectra of the dimer and other pertinent compounds are listed in Table I. On the basis of the similarity of the ultraviolet spectra of dianthracene and *o*-xylene, Coulson and co-workers concluded that dianthracene has the structure formed by bonding the *meso* atoms of two anthracene molecules (I).[3] This same similarity between the spectra of the photodimer of methyl β-naphthyl ether and *o*-xylene is noted except that in the case of the dimer a distinct bathochromic shift has taken place. Similar bathochromic shifts, attributable to methoxy groups, are observed on comparison of the



I

spectra of toluene and *m*-xylene with those of the corresponding methyl ethers (Table I). These spectral data strongly indicate that the dimer is formed by a 1,4–1,4 dimerization of the unsubstituted ring. The product II may have either of two steric configurations and may have either symmetrical or unsymmetrical disposition of the methoxyl groups with respect to each other. We have no way of further reducing the structural uncertainty at this time.



II

The infrared spectrum of the photodimer exhibits bands at 3010 and 3040 and at 1650 cm.$^{-1}$. Such bands have been attributed, respectively, to the C—H and C=C stretching modes of *cis*-olefins.[4]

Attempts to establish the structure more rigorously were foiled by the insolubility of the material and the fact that, in solution, it appears to revert rapidly to

(1) National Science Foundation Postdoctoral Fellow 1962–1963.

(2) See F. D. Greene, S. L. Misrock, and J. R. Wolfe, Jr., *J. Am. Chem. Soc.*, **77**, 3852 (1955), for a discussion and background concerning anthracene dimers.

(3) C. Coulson, L. E. Orgel, W. Taylor, and J. Weiss, *J. Chem. Soc.*, 2961 (1955).

(4) L. J. Bellamy, "The Infrared Spectra of Complex Molecules," John Wiley and Sons, Inc., New York, N. Y., 1958, p. 34.