sodium methoxide in 50 ml. of anhydrous refluxing xylene was added 50 ml. of a xylene solution containing 4.75 g. (0.02 mole) of (−)-1-methyl-3-benzoyl-3-chloropiperidine (IV). After refluxing for 30 minutes the mixture was cooled in a Dry Ice–acetone-bath and filtered through sintered glass. The solvent was removed under reduced pressure and replaced with 100 ml. of petroleum ether. This solution was treated with carbon and refiltered. Removal of the solvent gave a chlorine-free, colorless, dextrorotatory oil. The infrared spectrum showed no hydroxyl absorption, but did have a carbonyl absorption at 5.97 μ. In all other respects the spectrum was identical to that of 2-methoxy-2-phenyl-5-methyl-1-ox-5-azaspiro[2.5]octane.

Optical Stability of (−)-1-Methyl-3-benzoyl-3-chloropiperidine (IV) in Refluxing Anhydrous Xylene.—To 50 ml. of anhydrous refluxing xylene was added 0.463 g. (0.0019 mole) of (−)-1-methyl-3-benzoyl-3-chloropiperidine ($\alpha^{35}D$ (absolute ethanol) −5.0°). The solution was refluxed for 30 minutes, cooled, and extracted with 0.2 *N* hydrochloric acid. The acid aqueous was washed with petroleum ether, treated with excess sodium bicarbonate, and extracted with petroleum ether. The solvent was removed after dryng the extract over sodium sulfate, treating with carbon, and filtering through sintered glass. The resulting colorless oil showed an $\alpha^{35}D$ (absolute ethanol) −4.1° corresponding to 10% racemization.

The optical rotations were obtained using the conventional method as well as an analytical method based on the organic chlorine[23] content of an aliquot of the solutions taken for the polarimetric examinations.

Optical Stability of (+)-1-Methyl-3-benzoyl-3-hydroxypiperidine (VI) under the Conditions of the Quasi-Favorskii Rearrangement.—Optically active amine, $\alpha^{32}D$ (absolute ethanol) +10.6°, was recovered after subjection to the conditions described above for the quasi-Favorskii rearrangement. The optical rotation of the material was unchanged.

The Effect of Solvent Volume and Sodium Hydroxide Concentration on the Rearrangement of 1-Methyl-4-benzoyl-4-chloropiperidine.—The rearrangements were carried out as reported earlier.[1] In a series of three reactions 3.45 g. (0.0145 mole) of 1-methyl-4-benzoyl-4-chloropiperidine in 50 ml. of anhydrous petroleum ether, b.p. 125° (Skelly E), was added to 50, 200 or 200 ml., of Skelly E containing 3.50, 3.50 or 18.0 g. of finely powdered, dry (180° 0.1 mm.), 12 hours) sodium hydroxide (0.0875, 0.0875 and 0.450 mole), respectively. These reactions yielded 0.540, 0.191 and 0.730 g. of 1-methyl-4-carboxy-4-phenylpiperidine (0.00247, 0.00087 and 0.00333 mole) corresponding to 17, 6 and 23% yields, respectively.

MADISON, WISC.

[CONTRIBUTION FROM LEDERLE LABORATORIES, AMERICAN CYANAMID CO.]

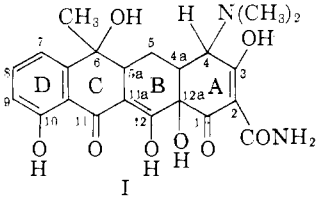
The 6-Deoxytetracyclines.[1] Further Studies on the Relationship between Structure and Antibacterial Activity in the Tetracycline Series

BY J. R. D. MCCORMICK, ELMER R. JENSEN, PHILIP A. MILLER AND ALBERT P. DOERSCHUK

RECEIVED NOVEMBER 13, 1959

The preparation by hydrogenolysis and the properties of 6-deoxytetracycline, 6-deoxy-6-demethyltetracycline and 6-deoxy-5-hydroxytetracycline are described. A description is given of a hydrogenation sequence starting with the tetracyclines and proceeding, apparently by way of the anhydrotetracyclines, to some 8-hydroxytetralone derivatives; some of these products are characterized. Relationships between structure and antibacterial activity in the tetracycline series are described.

Continuing work on the broad-spectrum antibiotic, tetracycline (I),[2-4] has shown that three changes can be made in the fundamental structure



I

of this compound with retention of its characteristic antimicrobial activity. For purposes of this study, a compound is considered to possess characteristic tetracycline activity if it exhibits as much as one-tenth the activity of tetracycline against all of a number of organisms in both *in vitro* and *in vivo* tests. These changes are replacement of 5-hydrogen by hydroxyl,[5] 7-hydrogen by chlorine[3,4] or bromine,[6] and 6-methyl by hydrogen.[7] The first two changes are in the direction of more complex structures, confer additional modes of instability on the molecule,[5,8,9] and give little information on the minimal structural requirements for antimicrobial activity. On the other hand, the third change, replacement of 6-methyl by hydrogen, produces a simplified molecule retaining high activity and possessing enhanced stability toward both acid and base.[7] Other localized changes in the tetracycline molecule, reported earlier and pertinent to the question of the structural requirements for activity, resulted in marked decreases in antimicrobial activity. These changes were (Table I): conversion of 2-carboxamide to nitrile,[10] replace-

(1) J. R. D. McCormick and E. R. Jensen, South African Patent 512/58, June 6, 1958; J. R. D. McCormick and E. R. Jensen, South African Patent 513/58, June 6, 1958. Abridgements of these patents were published in the *Union of South Africa Patent Journal*, issue of July 9, 1958, p. 22. A later preliminary communication of Stephens, *et al.* (THIS JOURNAL, **80**, 5324 (1958)) described two members of this series (6-deoxytetracycline and 6-deoxy-5-hydroxytetracycline).

(2) The trade mark of the American Cyanamid Co. for tetracycline is Achromycin.

(3) J. H. Boothe, J. Morton, J. P. Petisi, R. G. Wilkinson and J. H. Williams, THIS JOURNAL, **75**, 4621 (1953).

(4) L. H. Conover, W. T. Moreland, A. R. English, C. R. Stephens and F. J. Pilgrim, *ibid.*, **75**, 4623 (1953).

(5) 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, *ibid.*, **75**, 5455 (1953).

(6) P. Sensi, *Il. Farmaco Sci. Ed.*, **10**, 346 (1955); A. P. Doerschuk, 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, THIS JOURNAL, **78**, 1508 (1956).

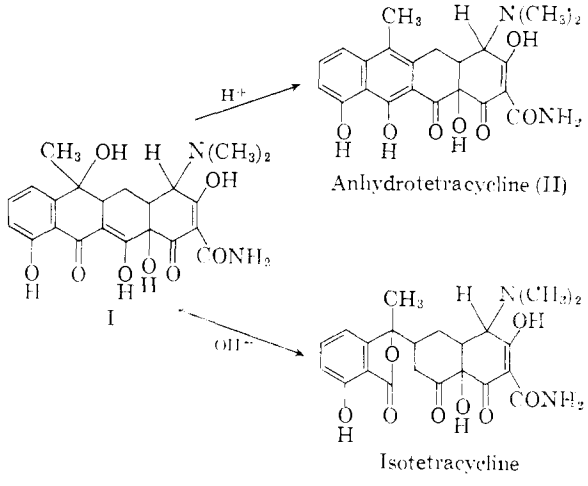
(7) J. R. D. McCormick, N. O. Sjolander, U. Hirsch, E. R. Jensen and A. P. Doerschuk, *ibid.*, **79**, 4561 (1957).

(8) 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).

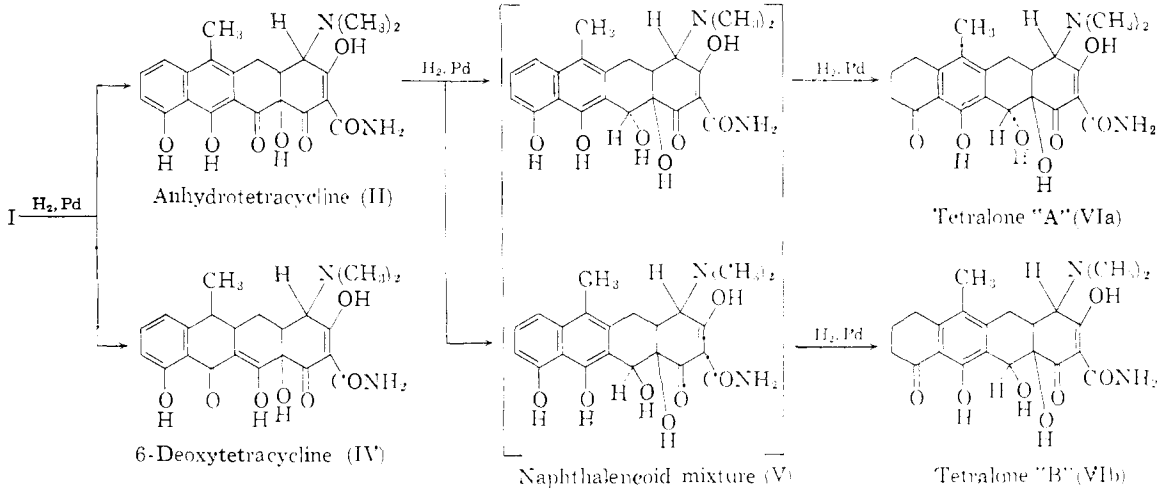
(9) C. W. Waller, B. L. Hutchings, C. F. Wolf, A. A. Goldman, R. W. Broschard and J. H. Williams, *ibid.*, **74**, 4981 (1952).

(10) Prepared from tetracycline by the method presented in ref. 12 for the preparation of 7-chlorotetracyclinonitrile.

ment of 4-dimethylamino by hydrogen,[11] conversion of 4-dimethylamino to methiodide,[12] inversion at C.4,[12] inversion at C.5a,[13] dehydration at C.5a,6,[5] dehydrogenation at C.5a,11a,[13] replacement of 12a-hydroxyl by hydrogen,[14] and the iso-rearrangement of ring C.[9]

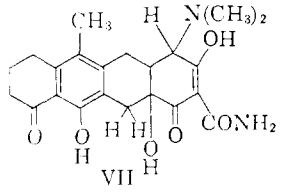


Hydrogenation of tetracycline under certain conditions yielded a complex mixture, one component of which was a tetracycline-like substance of R_f 0.70 in paper chromatographic system 2 (see Experimental). Isolation and characterization of this material showed it to be the desired 6-deoxytetracycline (IV). Other products of this reduction were components V whose gross ultraviolet absorption spectrum resembled that of 1,8-dihydroxynaphthalene. Reduction of anhydrotetracycline also gave rise to 1,8-dihydroxynaphthalene-like components. Upon more extensive reduction, both tetracycline and anhydrotetracycline yielded yet another mixture having ultraviolet absorption characteristics similar to those of 8-hydroxytetralone. Paper chromatographic examination of this latter mixture from either source showed it to contain two antibacterially inactive major components. Isolation of the hydrochloride of each of these two components from reduction mixtures made from anhydrotetracycline (II) showed that they were isomeric, $C_{22}H_{27}N_2O_7Cl$, and were essentially identical in ultraviolet absorption characteristics. Interpretation of these results was in terms of the following



This study deals with the replacement of 6-hydroxyl by hydrogen and the effect of this further simplifying change in structure on activity. This replacement is of particular interest in that it makes impossible the degradation of the tetracyclines to the anhydrotetracyclines or to the isotetracyclines; both these modes of degradation involve reaction of the 6-hydroxyl and have in the past represented a major barrier to the accomplishment of further localized structure modifications.[15]

A similar reduction sequence starting with tetracycline and leading to the closely related tetralone VII has been reported by Stephens, *et al.*[16]



VII

A notable feature of the sequence leading from tetracycline to VIa and VIb is that the action of catalyst and hydrogen is to remove 6-hydroxyl by dehydration rather than by hydrogenolysis. This dehydration reaction is normally acid-catalyzed but does not proceed at a measurable rate under the mildly acidic conditions of hydrogenolysis in the

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

(12) J. R. D. McCormick, S. M. Fox, L. L. Smith, B. A. Bitler, J. Reichenthal, V. E. Origoni, W. H. Muller, R. Winterbottom and A. P. Doerschuk, This Journal, **79**, 2849 (1957).

(13) J. R. D. McCormick, P. A. Miller, J. A. Growich, N. O. Sjolander and A. P. Doerschuk, *ibid.*, **80**, 5572 (1958).

(14) A. Green and J. H. Boothe, *ibid.*, in press.

(15) Even in the case of the relatively stable demethyltetracyclines, vigorous acid treatment results in dehydration in ring C to produce the corresponding anhydrotetracyclines (J. S. Webb, R. W. Broschard, D. B. Cosulich, W. J. Stein and C. F. Wolf, This Journal, **79**, 4563 (1957)).

(16) C. R. Stephens, K. Murai, H. H. Rennhard, L. H. Conover and K. J. Brunings, *ibid.*, **80**, 5324 (1958).

TABLE I

THE EFFECT OF CERTAIN LOCALIZED STRUCTURAL CHANGES ON THE *in Vitro* ANTIBACTERIAL ACTIVITY (*a*) OF TETRACYCLINE

	Relative activity, tetracycline = 100
Tetracycline	100
Tetracyclinonitrile	1
Dedimethylaminotetracycline	15[b]
Tetracycline methiodide	1
4-*epi*-Tetracycline	6
5-Hydroxytetracycline	80
5a-*epi*-Tetracycline	1
Anhydrotetracycline	6[a]
7-Chloro-5a(11a)-dehydrotetracycline	1
6-Demethyltetracycline	100
6-Deoxy-6-demethyltetracycline	200
6-Deoxytetracycline	70
6-Deoxy-5-hydroxytetracycline	50
7-Chlorotetracycline	350
7-Chloro-6-demethyltetracycline[c]	300
12a-Deoxytetracycline	1

[a] Activities were measured turbidimetrically (*Staph. aureus*) by a modification of the method of Pelcak and Dornbush (*Ann. N. Y. Acad. Sci.*, 51, 218 (1948),) the modification consisting of the addition of 10% normal horse serum to the test medium. It has been found that this modification yields results more closely paralleling those of *in vivo* tests. For example, the anhydrotetracycline family of compounds, while active in the unmodified test, shows little or no activity *in vivo* and in the modified *in vitro* test. [b] Dedimethylaminotetracycline represents an anomaly in this series in that, while showing significant *in vitro* activity, it shows no protective action in a number of experimental infections in rats (private communication, J. H. Boothe). It, therefore, does not possess characteristic tetracycline activity. [c] The trade mark of the American Cyanamid Co. for 7-chloro-6-demethyltetracycline is Declamycin.

absence of hydrogenation catalyst or of hydrogen. Promotion of other normally acid-catalyzed reactions under hydrogenation conditions has been previously noted.[17]

Analogous results were observed in the hydrogenation of 5-hydroxytetracycline and 6-demethyltetracycline.

Investigation of this new hydrogenation reaction led to a system which gave improved yields of 6-deoxytetracycline. The system was: tetracycline hydrochloride, 1 mole of boric acid,[18] sufficient hydrochloric acid to give a *p*H of 1.8, equal weight of 5% palladium-on-carbon, 10 volumes of 1:1 dimethylformamide–water, and contact with 3 atmospheres pressure of hydrogen at 25° for three hours. Through the reduction step a yield of 6% of 6-deoxytetracycline was obtained. Under these conditions 6-demethyltetracycline and 5-hydroxytetracycline were hydrogenolysed in 21 and 15% yields, respectively.

Structure assignments of the 6-deoxytetracyclines are based on composition, mode of formation, qualitative and quantitative changes in reactivity to acid and to alkali, ultraviolet absorption, and mobility and fluorescence characteristics on paper chromatograms. In the case of 6-deoxytetracycline

(17) For example: A. G. Caldwell and E. R. H. Jones (*J. Chem. Soc.*, 599 (1946)) have reported the Beckmann rearrangement of aldoximes under catalytic hydrogenation conditions.

(18) Boric acid served to modify the nature of the reaction by-products, simplifying analysis of the reaction mixtures and isolation of the 6-deoxytetracycline.

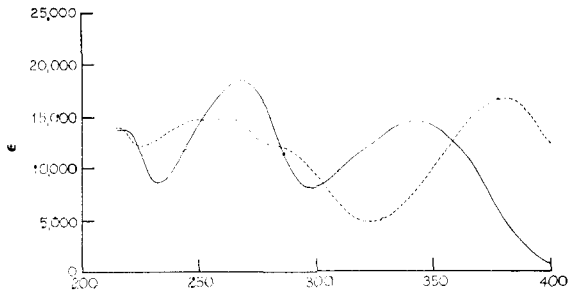


Fig. 1.—Ultraviolet absorption spectra of 6-deoxytetracycline in 0.1 *N* H_2SO_4 (——) and in 0.1 *N* NaOH (------).

itself, the composition of the hydrochloride, $C_{22}H_{24}N_2O_7 \cdot HCl$, indicated the loss of one oxygen from the parent, tetracycline. The mode of formation, though not definitive, represents the classical method for hydrogenolysis of benzylic hydroxyl.[19] The greatly increased stability to acid and to alkali, presented in Table II, together with the observation that the products of vigorous acidic and alkaline treatment do not belong to the anhydrotetracycline–isotetracycline families, is in full accord with the postulated loss of 6-hydroxyl. The characteristic tetracycline-like ultraviolet absorption spectra (Fig. 1) in acidic and in alkaline solutions demonstrate the close relationship of 6-deoxytetracycline to tetracycline and clearly differentiate it from the 12a-deoxytetracyclines.[14] The mobilities of 6-deoxytetracycline in a variety of partition

TABLE II

COMPARISON OF STABILITIES OF THE 6-DEOXYTETRACYCLINES AND THEIR PARENT TETRACYCLINES

	Half decomposition time[a] (minutes) in: 3 *N* HCl at 100°	0.1 *N* NaOH at 100°
6-Deoxytetracycline	1600	570
Tetracycline	≪1	6.8
6-Deoxy-6-demethyltetracycline	1600	200
6-Demethyltetracycline	1.4	32
6-Deoxy-5-hydroxytetracycline	2700	630
5-Hydroxytetracycline	≪1	2.2

[a] Determined as the rate of loss of the long wave length absorption maximum. In all cases, this loss has followed first-order kinetics for at least the first 30% of the reaction.

chromatographic systems were increased over those of tetracycline, in accord with the less polar nature expected (Table III). The ultraviolet fluorescence on paper chromatograms was red-orange, changing to intense green-yellow after exposure to ammonia, a response that experience in these laboratories has shown is closely related to an intact rings B–C–D chromophore system. Analogous observations were made for 6-deoxy-6-demethyltetracycline and 6-deoxy-5-hydroxytetracycline, establishing all the structures as presented.

The *in vitro* antibacterial activities of the 6-deoxytetracyclines can be classed as broad-spectrum, generally paralleling those of the previously known tetracyclines. Surprisingly, this simplication of the tetracycline molecule shows the

(19) "Organic Reactions," Vol. III, John Wiley and Sons, Inc. New York, N. Y., 1953, pp. 263–326.

TABLE III

CHROMATOGRAPHIC CHARACTERIZATION OF THE 6-DEOXYTETRACYCLINES AND THEIR C.4 EPIMERS

	% in equilibrium mixture	R_f, system 1[a]	R_f, system 2[a]
Tetracycline	55	0.03	0.47
6-Deoxytetracycline	50	.34	.70
6-Demethyl-6-deoxytetracycline	60	.26	.64
6-Deoxy-5-hydroxytetracycline	66	.10	.36
4-*epi*-6-Deoxytetracycline	50	.13	
4-*epi*-6-Demethyl-6-deoxytetracycline	40	.08	
4-*epi*-6-Deoxy-5-hydroxytetracycline	34	.10	

[a] These systems and their use are described in the Experimental portion.

6-hydroxyl group is not essential to broad-spectrum activity nor, indeed, as is seen in 6-deoxy-6-demethyltetracycline, is asymmetry at C.6 essential. In fact, quantitatively (Table IV), 6-deoxy-6-demethyltetracycline is the most active of the 6-deoxy-tetracyclines against *Staphylococcus aureus* (turbidimetric), having increased activity against this organism over that of the hydroxylated parent. These observations have carried over into preliminary *in vivo* experiments.[20]

TABLE IV

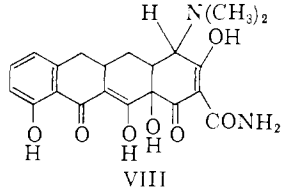
QUANTITATIVE COMPARISON OF *in Vitro* ANTIBACTERIAL ACTIVITIES[a] OF THE 6-DEOXYTETRACYCLINES AND THEIR PARENTS

	Relative activity, tetracycline = 100		Relative activity, tetracycline = 100
6-Deoxytetracycline	70	Tetracycline	100
6-Deoxy-6-demethyltetracycline	170	6-Demethyltetracycline	95
6-Deoxy-5-hydroxytetracycline	36	5-Hydroxytetracycline	100

[a] Activities were measured turbidimetrically (*Staph. aureus*) by the method of Pelcak and Dornbush, *Ann. N. Y. Acad. Sci.*, 51, 218 (1948).

Reversible conversion to the C.4 epimers, possessing diminished microbiological activities, has been observed for these 6-deoxytetracyclines.[12] The C.4 epimers are characterized by paper chromatographic R_f values in Table III.

6-Deoxy-6-demethyltetracycline (VIII) is the



VIII

simplest compound described that has been shown to have characteristic tetracycline antimicrobial activity as defined earlier. This compound has only four asymmetric centers, in contrast to six for 5-hydroxytetracycline. Of these four, only three represent potential problems for future synthetic work, since the fourth, that at C.4, has been previously shown to be readily controllable.[12]

(20) Private communication, H. J. White.

For two of these four centers, C.4 and C.5a, proper configuration has been previously shown to be essential for characteristic tetracycline activity.[12,13] Thus there remain only two asymmetric centers, those at C.4a and C.12a, where the effect of configuration on antibacterial activity is unresolved. The relative configurations at C.4a and C.12a have been indirectly established as those resulting in *cis*-A—B ring juncture.[21] Since these two asymmetric centers, together with that at C.5a, determine the gross shape of the molecule, it follows that a change in any one of these three centers would have a profound effect on molecular shape. Since it has been shown that inversion at one of these centers, C.5a, results in complete loss of activity,[13] it may be concluded that proper molecular shape, and therefore proper configuration at all of these three centers, is critical for activity. Accordingly, 6-deoxy-6-demethyltetracycline is of particular interest in that it may represent minimal asymmetry for tetracycline-like antibacterial actively.

Acknowledgment.—The authors wish to acknowledge, with appreciation, the work of A. C. Dornbush and staff for *in vitro* antibacterial activity comparisons, the work of L. Brancone and staff for microanalyses, the work of R. Livant for paper chromatographic characterizations, and the technical assistance of S. Nalesnyk.

Experimental

Hydrogenolyses.—All hydrogenolysis reactions were carried out as follows: two-hundredths mole of the tetracycline as the hydrochloride was dissolved in 100 ml. of 1:1 dimethylformamide–water and to this was added 1.2 g. (0.02 mole) of boric acid, 10 g. of 5% palladium-on-carbon hydrogenation catalyst, and sufficient concentrated hydrochloric acid to bring the *p*H to 1.8. The mixture was contacted with hydrogen in the Parr low-pressure hydrogenation apparatus until a molar equivalent amount of hydrogen had been absorbed (usually about 2 hours). The catalyst was then removed by filtration and the resulting product, called the reduced solution in the followir g, was worked up as described below for the individual products.

6-Deoxy-6-demethyltetracycline (VIII).—Reduced solution representing 9.35 g. (0.02 mole) of 6-demethyltetracycline hydrochloride and containing, by microbiological assay, 1.87 g. (21%) of 6-demethyl-6-deoxytetracycline was concentrated *in vacuo* to a heavy sirup. To this was added 50 ml. of water and the resulting slurry was freeze-dried. The dry product was taken up in 100 ml. of methanol (not completely soluble); 100 ml. of absolute ether was added and the insolubles separated by centrifugation. The supernatant clear solution was evaporated to about 60 ml. in a stream of dry nitrogen, 0.5 ml. of concentrated hydrochloric acid was added, and the evaporation continued to a volume of 4.0 ml. Crystallization was initiated by scratching with a glass rod. After aging at room temperature for 18 hours with occasional stirring, the crystalline product was collected by filtration, washed with methanol–ether, and dried *in vacuo* at 40°. The product (540 mg., 6% yield) was recrystallized from ethanol as the hydrochloride to constant ultraviolet absorption properties, by dissolving with triethylamine and reprecipitating with concentrated hydrochloric acid: $[\alpha]^{25}D$ −109° (0.5% in 0.1 *N* H_2SO_4), m.p. (block) dec. 215–220°. The ultraviolet absorption spectrum in 0.1 *N* H_2SO_4 (aq.) showed λ_{max} in mμ (ε): 217 (13,400), 268 (19,000), 343 (14,600).

Anal. Calcd. for $C_{21}H_{22}N_2O_7 \cdot HCl \cdot {}^1/_2H_2O$: C, 54.90; H, 5.26; N, 6.10; O, 26.10; Cl, 7.72; H_2O, 1.96. Found: C, 54.81; H, 5.32; N, 6.16; O, 25.91 (difference); Cl, 7.80; H_2O (loss on drying), 2.06.

(21) L. H. Conover, "Symposium on Antibiotics and Mould Metabolites" Special Publication No. 5, The Chemical Society, London, pp. 48–81.

6-Deoxytetracycline.—Reduced solution prepared as above from 9.63 g. (0.02 mole) of tetracycline hydrochloride and containing 0.58 g. (6%) of 6-deoxytetracycline was adjusted to *p*H 3.0 with ammonium hydroxide and evaporated to dryness *in vacuo* at 25–30°. The resulting solid product was leached twice with 100-ml. portions of water-saturated butanol, and the combined butanol extract was concentrated *in vacuo* at 30° with water addition to produce 50 ml. of an aqueous concentrate. The concentrate was adjusted to *p*H 3.0 with ammonium hydroxide, precipitating solid material which was filtered off. The filtrate was extracted with two 30-ml. portions of butanol; the butanol extracts were combined, adjusted to *p*H 1.5 with concentrated hydrochloric acid, and concentrated *in vacuo* to about 4 ml. Crystallization of 6-deoxytetracycline occurred during the concentration. The crystalline product was collected by filtration, washed with butanol and ether, and dried; yield 185 mg. (2%). The product was recrystallized to constant spectrophotometric properties by dissolving in butanol with triethylamine and reprecipitating the hydrochloride by the use of hydrochloric acid: $[\alpha]^{25}D$ −292° (0.5% in 0.1 *N* H_2SO_4), m.p. dec. 220–245°. The ultraviolet absorption spectrum in 0.1 *N* H_2SO_4 (aq.) showed λ_{max} (ϵ): 218 (13,200), 268 (18,600), 344 (14,700).

Anal. Calcd. for $C_{22}H_{24}N_2O_7 \cdot HCl$: C, 56.90; H, 5.42; N, 6.02; Cl, 7.63; O, 24.1. Found: C, 56.96; H, 5.38; N, 5.79; Cl, 7.63; O, 24.2 (difference).

6-Deoxy-5-hydroxytetracycline Hydrochloride.—Reduced solution prepared as above from 9.94 g. (0.02 mole) of 5-hydroxytetracycline hydrochloride and containing 1.49 g. (15%) of 6-deoxy-5-hydroxytetracycline was adjusted to *p*H 3.0 with ammonium hydroxide and concentrated to dryness *in vacuo* at 25–30°. The resulting solid product was leached twice with 75-ml. portions of water by stirring for 1 hour at 25°. The extracts were combined and found to contain about 1.10 g. of 6-deoxy-5-hydroxytetracycline (spectrophotometric assay). This solution was adjusted to *p*H 1.0 with hydrochloric acid and extracted twice with equal volumes of butyl alcohol. The combined butyl alcohol extract was concentrated *in vacuo* with water addition to yield 50 ml. of aqueous concentrate which was then freeze-dried. The freeze-dried product was taken up in acetone by the addition of concentrated hydrochloric acid, and filtrated to remove some ammonium chloride; 6-deoxy-5-hydroxytetracycline hydrochloride was crystallized from the filtrate by seeding. The crude product, 450 mg. (6.5% recovery), was recrystallized twice to constant properties (ultraviolet absorption) as described for 6-deoxytetracycline hydrochloride; 6-deoxy-5-hydroxytetracycline hydrochloride: $[\alpha]^{25}D$ −251° (0.5% in 0.1 *N* H_2SO_4), m.p. (block) dec. 230–250°. The ultraviolet absorption spectrum in 0.1 *N* H_2SO_4 (aq.) showed λ_{max} (ϵ): 218 (14,000), 267 (19,100), 340 (15,100).

Anal. Calcd for $C_{22}H_{24}N_2O_8 \cdot HCl$: C, 54.9; H, 5.20; N, 5.83; Cl, 7.38; O, 26.70. Found: C, 54.48; H, 4.81; N, 5.50; Cl, 7.74; O (Unterzaucher), 26.55.

Epimerization of 6-Deoxytetracyclines.—Equilibration solvent was prepared by diluting one molar aqueous NaH_2PO_4 with two volumes of methanol.[12] To 200 ml. of this solvent there was added 1 g. of 6-deoxytetracycline and the solution was allowed to stand at 25° under nitrogen for 24 hours. Quantitative paper chromatographic examination of the solution then showed that epimeric equilibration had occurred, the mixture containing about 50% of the new C.4 epimer. The R_f in system 2 was 0.13; the R_f of 6-deoxytetracycline was greater than 0.9. Parallel experiments starting with 6-deoxy-6-demethyltetracycline and with 6-deoxy-5-hydroxytetracycline showed analogous results. The equilibrium compositions and chromatographic R_f's are summarized in Table III.

Reduction of Anhydrotetracycline to Tetralone A (VIa) and Tetralone B (VIb) and to "Naphthaleneoid Mixture" (V).—To five grams of anhydrotetracycline hydrochloride there was added 120 ml. of a 1:1 mixture of dimethylformamide and water. The solution was adjusted to *p*H 7.4 with sodium hydroxide and to this was added 5 g. of 5% palladium-on-carbon hydrogenation catalyst. The mixture was contacted with hydrogen at 45 p.s.i.g. for 1.75 hours, at which time two equivalents of hydrogen had been absorbed. The mixture was filtered and the filtrate examined by spectrophotometric and paper chromatographic means. The ultraviolet absorption showed maxima at 266 and 345 mμ and indicated a 55% yield of tetralones when calculated as tetralone A. Paper chromatographic analysis (system 3) showed the presence of both tetralone A (R_f 0.40) and tetralone B (R_f 0.75), the former component predominating. For isolation of the tetralone products, the filtered reduction mixture was concentrated *in vacuo* to a thick sirup, diluted with 100 ml. of water, and freeze-dried to remove residual dimethylformamide. The resulting solid (3.5 g.) was dissolved in 50 ml. of water-saturated butanol and chromatographed on a 3-inch column using the 80:20 butanol–chloroform, 0.01 *N* hydrochloric acid system previously described.[12] One-hundred ml. cuts of the butanol–chloroform eluate were collected and examined by paper chromatographic analysis (system 3). Cuts 3, 4 and 5 from the column were seen to consist of only one component, a yellow-fluorescing band on the papergrams at R_f 0.75 (tetralone B). These rich cuts were combined, concentrated *in vacuo* with water addition, and the aqueous concentrate freeze-dried. The amorphous product was crystallized as the hydrochloride from acetone–hydrochloric acid; yield 144 mg. of white needles, $[\alpha]^{25}D$ + 146° (0.5% in 0.1 *N* H_2SO_4), m.p. 200–205° dec.; ultraviolet absorption spectrum in 0.1 *N* H_2SO_4 (aq.), λ_{max} (ϵ): 220 mμ (20,000), 232 mμ (infl.) (18,800), 266 mμ (28,800), 346 mμ (3,900).

Anal. Calcd. for $C_{22}H_{26}N_2O_7 \cdot HCl$: C, 56.6; H, 5.83; N, 6.00; Cl, 7.60. Found: C, 57.0; H, 6.34; N, 5.74; Cl, 7.62.

Tetralone A was observed as the principal component in column chromatographic cuts 7 through 19 by its characteristic yellow fluorescence on papergrams at R_f 0.40. Isolation of tetralone A hydrochloride was accomplished exactly as indicated for tetralone B above. The yield was 170 mg. of white needles, $[\alpha]^{25}D$ (0.5% in 0.1 *N* H_2SO_4) +176°, m.p. dec. 195–210°. The ultraviolet absorption spectrum in 0.1 *N* H_2SO_4 showed λ_{max} (ϵ): 220 mμ (17,800), 235 mμ (18,000), 266 mμ (28,100), 343 mμ (4,320).

Anal. Calcd. for $C_{22}H_{26}N_2O_7 \cdot HCl$: C, 56.6; H, 5.83; N, 6.00; Cl, 7.60. Found: C, 56.4; H, 6.19; N, 5.68; Cl, 7.54.

Less extensive reduction of anhydrotetracycline under the same conditions (*e.g.*, 30 minutes contact, about 1.1 moles hydrogen absorbed) resulted in "naphthaleneoid mixture" (V) as judged from the characteristic absorption spectrum, λ_{max} 313, 327, 342 mμ, exactly as is indicated below for reduction of tetracycline itself.

Reduction of Tetracycline to "Naphthaleneoid Mixture" (V) and to Tetralones A (VIa) and B (VIb).—Five grams of tetracycline hydrochloride was dissolved in 100 ml. of 1:1 dimethylformamide–water. Five grams of 5% palladium-on-carbon catalyst was added and the mixture contacted with hydrogen until about one mole of hydrogen had been absorbed (64 min.). The ultraviolet absorption spectrum of the resulting solution showed strong fine structure (λ_{max} 313, 327, 342 mμ), characteristic of the 1,8-dihydroxynaphthalenes. Attempted isolation of the product(s) responsible for this characteristic absorption showed that these products were very labile, the fine structure in the spectrum disappearing completely after a few hours contact with air. More extensive reduction of tetracycline under the same conditions (*e.g.*, 20 hours reduction time, about 2 moles of hydrogen taken up) resulted in the production of tetralones A and B, exactly as from the reduction of anhydrotetracycline. This was shown by close similarity of the absorption spectrum of the reduction mixture (λ_{max} 266 and 345 mμ) and of the papergram results (yellow fluorescing zones (system 3) at R_f 0.40 and at 0.75).

Paper Chromatography.—All paper chromatographic examinations were carried out using buffered $7^1/_2 \times 22$ inch sheets of Whatman No. 1 paper. Up to six samples were spotted on each sheet, the loading being about 10 to 25 mcg. per sample. The sheets were developed by the descending technique after a preliminary equilibration with the developing solvent vapor in the chromatographic jar. Three systems were used as follows:

System 1.—In this system, all operations were conducted at 4°. One liter of a mixture of one part butanol and 3 parts chloroform was equilibrated with 500 ml. of aqueous buffer consisting of 0.3 *M* NaH_2PO_4 adjusted to *p*H 2.0 with 85% phosphoric acid. Whatman No. 1 paper sheets were dipped in the aqueous phase and dried. A shallow 6-inch dish of the organic phase was placed on the bottom of

a 12- by 24-inch round chromatographic jar and the floor of the jar was flooded with the equilibrated aqueous phase. The required number of buffered sheets were placed in the jar and the jar tightly covered for 16 hours. The samples were applied to the pre-marked sheets by means of a pipet inserted through a hole in the rotatable cover. The developing solvent (butanol–chloroform) was added and the development carried out over a 4- to 5-hour period. After development, the sheets were removed from the jar, air-dried in the dark, and inspected by light and under ultraviolet radiation before and after exposure to ammonia.

System 2.—All operations were carried out at 25 ± 0.5°. Ethyl acetate was equilibrated by shaking with an equal volume of McEvain's *p*H 4.5 buffer. The chromatographic sheets were dipped in the aqueous phase and dried. The solvent and aqueous phases were placed separately in the bottom of a 12 by 24 inch jar as described for system 1. Up to four buffered sheets were placed in the jar and allowed to equilibrate with the solvent atmosphere for 16 hours. After this paper pre-equilibration, the samples were spotted onto pre-marked locations across the tops of the sheets by means of a pipet inserted through a small hole in the rotatable lid of the jar. The ethyl acetate phase was then added to the top trough to begin development; about 4 hours development time was required for solvent travel of 18 inches.

System 3.—All operations were carried out at 25 ± 0.5°. One liter of butanol was equilibrated with 150 ml. of aqueous 0.3 *M* NaH_2PO_4 which had been adjusted to *p*H 3.0 with 85% phosphoric acid. The chromatographic sheets were dipped in the aqueous phase and dried. The chromatographic jar was arranged for descending development and the bottom of the jar was flooded with butanol-saturated water. Dry sheets were spotted with sufficient sample to contain 10 to 25 mcg. of tetracycline derivative. These sheets were then placed in the jar and pre-equilibrated with the jar atmosphere for 60 min. The butanol phase was used for development, about 16 hours being required for 18-inch flow. After development, the sheets were removed and examined as described above.

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Imidazole Catalysis. VII. The Dependence of Imidazole Catalysis of Ester Hydrolysis on the Nature of the Acyl Group[1]

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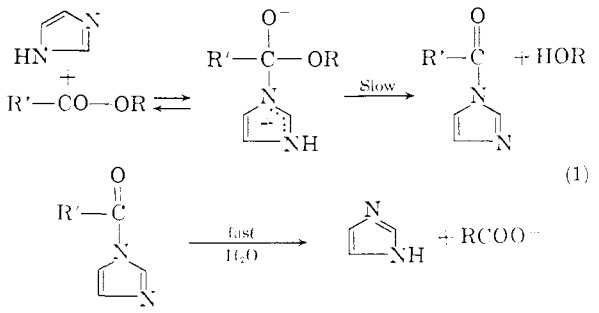
RECEIVED JANUARY 6, 1960

A new synthesis of 4-(2′-hydroxyphenyl)-imidazole (IV) is described. A number of substituted O-benzoyl esters of IV have been prepared and their solvolysis with intramolecular imidazolyl participation (30°) studied. From the Hammett *ρ*-constant it is concluded that the rate of imidazole catalyzed hydrolysis of substituted phenol esters is equally sensitive to the electronic nature of the ester carbonyl group and the leaving tendency of the phenoxide ion. Several aliphatic esters of 4-(2′-hydroxyethyl)-imidazole were also prepared and their rates of solvolysis with intramolecular imidazolyl participation as well as by specific base catalysis have been determined (78°). The results of this study indicate that the imidazolyl group, though many powers of 10 a weaker base than alkoxide, is capable of displacing the latter to bring about the catalysis of the hydrolysis of aliphatic esters in intramolecular processes. The values of the various rate constants for hydrolysis of the aliphatic esters are discussed in terms of inductive and electrostatic effects.

Introduction

In previous studies the nucleophilic catalysis of ester hydrolysis by imidazoles has been shown to be quite sensitive to the leaving tendency of the -OR group.



Thus, for the bimolecular catalysis of the hydrolysis of *m*- and *p*-substituted phenyl acetates the *ρ*-for imidazole,[1a] 4-hydroxymethyl imidazole[1b] and histidine[1b] are between 1.7 and 1.9 whereas the *ρ*-for hydroxide ion catalysis is but 1.0.[1a,3] Also, though imidazole is capable of displacing aliphatic thiol from acyl thiols both bimolecularly[4] and intramolecularly,[1f] the displacement of alkoxide in the bimolecular process has never been observed and occurs only with difficulty in the more efficient intramolecular displacement reaction .[1e] These results have been rationalized on the basis of a competition between the attacking base and the potential leaving group for elimination from the tetrahedral intermediate. It has been suggested that the group which departs most readily is the one whose conjugate acid possesses the lowest pK_a (quantitatively applicable in a given series of like substituents, as *e.g.* in the case of substituted phenoxides, but grossly so in general).[1a,5]

To obtain a clearer picture of the transition state barriers in imidazole catalyzed ester hydrolysis, it was considered desirable to obtain information on the sensitivity of the rate constants to alterations of the electronic properties of the ester carbonyl group. The assessment of this factor along with some observations on the influence of electrostatic charge on the intramolecular catalysis of aliphatic ester hydrolysis is reported in this paper.

(1) For previous papers in this series see: (a) T. C. Bruice and G. L. Schmir, THIS JOURNAL, **79**, 1663 (1957); (b) *ibid.*, **80**, 148 (1958), (c) G. L. Schmir and T. C. Bruice, *ibid.*, **80**, 1173 (1958); (d) T. C. Bruice and R. Lapinski, *ibid.*, **80**, 2265 (1958); (e) T. C. Bruice and J. M. Sturtevant, *ibid.*, **81**, 2860 (1959); (f) T. C. Bruice, *ibid.*, **81**, 5444 (1959).

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(3) T. C. Bruice and M. F. Mayahi, THIS JOURNAL, **82**, 3067 (1960).

(4) M. L. Bender and B. W. Turnquest, *ibid.*, **79**, 1652, 1656 (1957).

(5) K. B. Wiberg, *ibid.*, **77**, 2519 (1955).