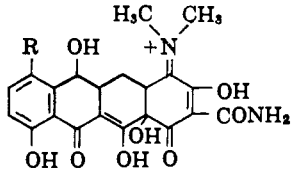
had the characteristic spectral properties of II but an analysis ($\lambda_{max}^{MeOH,\ 0.1\ N\ HCl}$ 258 and 336 mμ (log ε 4.36 and 3.70); λ_{max}^{KBr} 5.83. *Anal.* Found for $C_{21}H_{20}N_2O_8$: C, 58.75; H, 4.86; N, 6.46) revealed that the dimethylamino group was still present. Mild acid hydrolysis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



X, R = H or Cl

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

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

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

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

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

PHARMACEUTICAL PRODUCT DEVELOPMENT SECTION
LEDERLE LABORATORIES
PEARL RIVER, NEW YORK

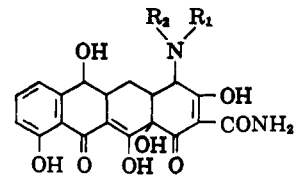
ROBERT C. ESSE
J. A. LOWERY
C. RICHARD TAMORRIA
G. M. SIEGER

RECEIVED JULY 1, 1964

Tetracycloxides. II. Transformations at the C-4 Position

Sir:

It was noted in the accompanying report[1] that 4-hydroxytetracycloxide can be reductively aminated catalytically with methylamine to give 4-dedimethylamino-4-methylamino-6-demethyltetracycline (II) and that this product can, in turn, be reductively methylated with formaldehyde to yield 6-demethyltetracycline (VIII). The reductive amination step has been extended to include the use of ammonia and a number of simple aliphatic primary amines. The resulting derivatives are summarized in Table I. The reductive aminations were carried out at room temperature at pressures of 50 lb. or less, with 10% palladium on carbon as the catalyst. Generally, 10 equiv. of amine was used. The large excess of amine served two purposes; it afforded the alkaline conditions necessary for the success of the reaction and it minimized the formation of 4-dedimethylamino-4-hydroxy-6-demethyltetracycline.[2] These reactions were rapid, being complete within 20 min. Even so, there was a considerable loss in side reactions which stems from the great alkaline instability of 4-hydroxytetracycloxide. Rapid handling of the reaction solution prior to commencing hydrogenation was essential to minimize losses. The yields of crystalline product isolated ranged from 20 to 40%.



I-XII

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

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

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

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

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

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

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

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

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

TABLE I

	R_1	R_2	Compound	Reaction solvent	Amine used	Composition	Analyses, % C	H	N	R_f values in paper chromatographic systems System A[a]	System B[a]
I[b]	H	H	4-Dedimethylamino-4-amino-6-demethyltetracycline	2-Methoxyethanol	Ammonia	$C_{19}H_{18}N_2O_8 \cdot CH_3OH$	55.15	5.12	6.67	0.03	0.11
II	H	CH_3	4-Dedimethylamino-4-methylamino-6-demethyltetracycline	2-Methoxyethanol	Methylamine	$C_{20}H_{20}N_2O_8 \cdot H_2O$	55.74	5.38	6.35	0.03	0.17
III[c]	H	C_2H_5	4-Dedimethylamino-4-etlylamino-6-demethyltetracycline	Tetrahydrofuran	Ethylamine	$C_{21}H_{22}N_2O_8 \cdot H_2O$	56.69	5.52	6.20	0.09	0.28
IV	H	*n*-C_3H_7	4-Dedimethylamino-4-*n*-propylamino-6-demethyltetracycline	Tetrahydrofuran	*n*-Propylamine	$C_{22}H_{24}N_2O_8$	59.16	5.77	6.67	0.30	0.47
V	H	*n*-C_4H_9	4-Dedimethylamino-4-*n*-butylamino-6-demethyltetracycline	Tetrahydrofuran	*n*-Butylamine	$C_{23}H_{26}N_2O_8$	60.19	5.62	6.07	0.54	0.57
VI	H	C_2H_4OH	4-Dedimethylamino-4-(2-hydroxyethyl)-amino-6-demethyltetracycline	Methanol	2-Aminoethanol	$C_{21}H_{22}N_2O_9$	56.01	5.39	5.81	0.07	0.12
VII	H	C_3H_6OH	4-Dedimethylamino-4-(3-hydroxypropyl)amino-6-demethyltetracycline	Methanol	3-Aminopropanol	$C_{22}H_{24}N_2O_9$	57.16	5.44	5.91	0.04	0.15

[a] System A: methyl ethyl ketone–water, paper treated with 0.1 *M* Versene at pH 7.7. System B: butanol–water, paper treated with 0.1 *M* NaH_2PO_4 adjusted to pH 2.0 with HCl. [b] A superior preparation of I is by catalytic reduction of the 4-oxime analog. [c] The ultraviolet spectrum of III ($\lambda_{max}^{0.1\ N\ HCl}$ 258 and 355 mμ (log ε 4.27 and 4.15)) is typical for this class of compound.

TABLE II

	R_1	R_2	Compound	Compound alkylated	Aldehyde used	Reaction solvent	Composition	Analyses, % C	H	N	R_f values in paper chromatographic systems System A[a]	System B[a]
VIII	CH_3	CH_3	6-Demethyltetracycline	II	Formaldehyde	2-Methoxyethanol	$C_{21}H_{22}N_2O_8$				0.27	0.12
IX	CH_3	C_2H_5	4-Dedimethylamino-4-methylethylamino-6-demethyltetracycline	III	Formaldehyde	Methanol	$C_{22}H_{24}N_2O_8 \cdot H_2O$	57.44	5.92	6.07	0.45	0.18
X	CH_3	C_3H_7	4-Dedimethylamino-4-methylpropylamino-6-demethyltetracycline	IV	Formaldehyde	Methanol	$C_{23}H_{26}N_2O_8 \cdot HCl$[b]				0.78	0.37
XI	CH_3	C_2H_4OH	4-Dedimethylamino-4-methyl(2-hydroxyethyl)-amino-6-demethyltetracycline	VI	Formaldehyde	Methanol	$C_{22}H_{24}N_2O_9 \cdot CH_3OH$	56.56	5.75	5.65	0.42	0.12
XII	C_2H_5	C_2H_5	4-Dedimethylamino-4-diethylamino-6-demethyltetracycline	III	Acetaldehyde	Dioxane	$C_{23}H_{26}N_2O_8$	60.36	5.87	5.89	0.63	0.28

[a] See footnote *a*, Table I. [b] Purified by partition chromatography, not analyzed.

TABLE III

In Vitro ANTIBACTERIAL ACTIVITIES[6]

Compound	Relative activity (tetracycline = 100)
VIII	96
IX	75
X	50
XI	12
XII	25

cycline. The 4-dialkylamino analogs retain much of the activity of the parent tetracycline. The data in Table III show a trend to diminished activity as the bulkiness of the C-4 substituent increases.

The tetracycloxides permit modification at skeletal carbon on a scale previously realized only at the benzylic C-6 position[7] and the aromatic C-7 and C-9 positions.[8] It has been known for some time that 4-dedimethylamino tetracyclines, 4-*epi*-tetracyclines, and quaternized tetracyclines have little tetracycline-like antimicrobial properties.[9] The present study defines,

(7) C. R. Stephens, J. J. Beereboom, H. H. Rennhard, P. N. Gordon, K. Murai, R. K. Blackwood, and M. Scach von Wittenau, *J. Am. Chem. Soc.*, **85**, 2643 (1963); R. K. Blackwood, J. J. Beereboom, H. H. Rennhard, M. Scach von Wittenau, and C. R. Stephens, *ibid.*, **85**, 3943 (1963).

(8) J. H. Boothe, J. J. Hlavka, J. P. Petisi, and J. L. Spencer, *ibid.*, **82**, 1253 (1960).

(9) J. R. D. McCormick, E. R. Jensen, P. A. Miller, and A. P. Doerschuk, *ibid.*, **82**, 3381 (1960).

more precisely, the structural requirements at C-4 for antibiotic activity.

PHARMACEUTICAL PRODUCT DEVELOPMENT SECTION
LEDERLE LABORATORIES
PEARL RIVER, NEW YORK

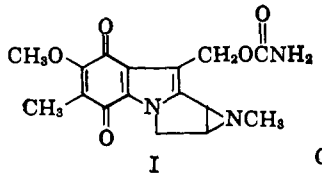
ROBERT C. ESSE
J. A. LOWERY
C. RICHARD TAMORRIA
G. M. SIEGER

RECEIVED JULY 1, 1964

The Mitomycin Antibiotics. Synthetic Studies. II.[1] The Synthesis of 7-Methoxymitosene, an Antibacterial Agent

Sir:

During their research on the structure of the mitomycin class of antibiotics, Patrick, Webb, and coworkers[2] isolated an aziridinopyrrolo[1,2-*a*]indoloquinone which was shown to have structure I and was found to be an orally active antibacterial agent of considerable interest. In the present communication we describe the preparation and antibacterial properties of the related 7-methoxymitosene[3] (II, 2,3-dihydro-9-hydroxymethyl-7-methoxy-6-methyl-1H-pyrrolo[1,2-*a*]indole-5,8-dione carbamate).



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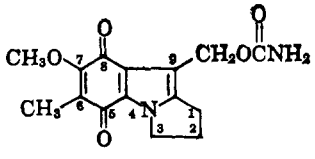


II

4-Nitro-2,5-xylenol[5] was converted *via* the methyl ether [m.p. 91–92°, λ_{max} 312 mμ (ε 7600)][6] by the Reissert technique[7] into 5-methoxy-6-methyl-2-indolecarboxylic acid (III) [m.p. 240–241° (gas), λ_{max} 294 mμ (ε 18,400)]. On treatment with potassium *t*-butoxide and methyl acrylate, the methyl ester of III [m.p. 149–150°, λ_{max} 298 mμ (ε 19,900)] furnished the β-ketoester IV [m.p. 180–182°, λ_{max} 336 mμ (ε 21,800)]. Acid-catalyzed decarbomethoxylation of IV then gave the tricyclic ketone V [m.p. 213–215°, λ_{max} 331 mμ (ε 21,200)].[8] Wolff–Kishner reduction of V gave pyrrolo[1,2-*a*]indole (VI) [m.p. 116–118°, λ_{max} 279 (ε 7930), 295 (ε 6930), and 308 mμ (ε 4530)] which was formylated[9] (Villsmeier–Haack) giving aldehyde VII [m.p. 187–189°, λ_{max} 256 (ε 18,200), 282 (ε 16,800) and 309 mμ (ε 13,500)].

This aldehyde was the key intermediate in the synthesis of II, for in addition to possessing the fundamental pyrrolo[1,2-*a*]indole ring system, the 7-methoxy function represented an entry for the elaboration of the 7-methoxy-5,8-pyrrolo[1,2-*a*]indoloquinone system[1] and the conversion of a β-indolecarboxaldehyde into the corresponding carbinol carbamate had already been demonstrated.[10]

Cleavage of the methoxy group in VII with aluminum chloride in refluxing xylene[11] gave the phenolic aldehyde VIII [m.p. >300°, λ_{max} 256 (ε 15,910), 283 (ε 14,910), and 311 mμ (ε 13,000)] which on oxidation with potassium nitrosodisulfonate[12] afforded the *o*-quinone IX [m.p. 240–248° dec., λ_{max} 225 (ε 86,700), 280 (ε 22,500), and 345 mμ (ε 11,400)]. Thiele acetoxylation of this quinone furnished the triacetate X [m.p. 264–265°, λ_{max} 218 (ε 28,000), 248 (ε 18,300), and 305 mμ (ε 11,200)], which on alkaline hydrolysis followed by air oxidation afforded the 7-hydroxy-5,8-pyrrolo[1,2-*a*]indoloquinone (XI) [m.p. 219–221°; λ_{max} 219 (ε 21,300), 299 (ε 14,450), and 330 mμ (ε 8100); $\lambda_{max}^{0.1\,N\,NaOH}$ 236 (ε 23,800), 299 (shoulder, ε 13,000), and 325 mμ (ε 13,700)]. Methylation (diazomethane) of XI gave methoxyquinone XII [m.p. 224–227°, λ_{max} 216 (ε 25,000), 243 (ε 14,900), 272 (ε 14,250), 289 (ε 13,870), and 332 mμ (ε 7120)].

Elaboration of the carbamate side chain from the quinone aldehyde XII was achieved in the following manner. Reduction of XII with sodium borohydride[13] followed by oxidation of the intermediate hydroquinonecarbinol with acidic ferric chloride[14] afforded the quinonecarbinol XIII [m.p. 180–182°, λ_{max} 230 (ε 17,700), 287 (ε 13,600), 350 (ε 3340), and 460 mμ (ε 1990)].[15] Acylation of this carbinol in pyridine with phenyl chloroformate gave the phenyl carbonate XIV [m.p. 137.5–138.0°, λ_{max} 230 (ε 19,050), 285 (ε 13,900), 345 (ε 3800), and 450 mμ (ε 950)] which on ammonolysis[16] was converted into 7-methoxymitosene (II) [m.p. 206–207°, λ_{max} 230 (ε 19,200), 287 (ε 14,600), 345 (ε 3870), and 460 mμ (ε 1390)].

(1) For paper I see W. A. Remers, P. N. James, and M. J. Weiss, *J. Org. Chem.*, **28**, 1169 (1963).

(2) J. B. Patrick, R. P. Williams, W. E. Meyer, W. Fulmor, D. B. Cosulich, R. W. Broschard, and J. S. Webb, *J. Am. Chem. Soc.*, **86**, 1889 (1964).

(3) Mitosene is the trivial name that has been proposed[4] for the structure 2,3-dihydro-9-hydroxymethyl-6-methyl-1H-pyrrolo[1,2-*a*]indole-5,8-dione carbamate.

(4) J. S. Webb, D. B. Cosulich, J. H. Mowat, J. B. Patrick, R. W. Broschard, W. E. Meyer, R. P. Williams, C. F. Wolf, W. Fulmor, C. Pidacks, and J. E. Lancaster, *J. Am. Chem. Soc.*, **84**, 3185 (1962).

(5) K. Auwers and F. Michaelis, *Ber.*, **47**, 1289 (1914).

(6) All compounds, except ketone V, gave satisfactory analyses; infrared spectra were in accord with the assigned structures. Ultraviolet spectra are for methanol solutions, except where otherwise noted.

(7) P. L. Julian, E. W. Meyer, and H. C. Printy in "Heterocyclic Compounds," Vol. 3, R. C. Elderfield, Ed., John Wiley and Sons, Inc., New York, N. Y., 1952, p. 18.

(8) For the p.m.r. spectrum of V, see spectrum No. 299 in "NMR Spectra Catalog," Varian Associates, Palo Alto, Calif., 1962.

(9) W. A. Remers, R. H. Roth, and M. J. Weiss, *J. Am. Chem. Soc.*, in press.

(10) The essential requirement for success in this conversion is the presence of an appropriate electronegative substituent which affords stabilization of the 3-indolylmethanol system and its derivatives (see ref. 15).

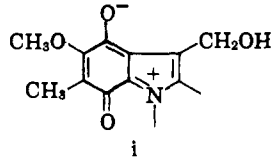
(11) The stability of the 9-formyl group to these cleavage conditions had been demonstrated previously with a model system by Dr. Remers.

(12) H. Teuber and G. Thaler, *Ber.*, **91**, 2253 (1958), and previous papers.

(13) *Cf.* E. Leete, *J. Am. Chem. Soc.*, **81**, 6023 (1959), and references cited therein.

(14) Earlier attempts to regenerate the quinone from this intermediate by air oxidation were not successful (*cf.* the conversion with air of the 9-aldehyde X to XI). We interpret this difference in behavior toward oxygen to be the result of the reduced nucleophilicity of C-9 in the 9-aldehyde series. [The facile reaction of 3-alkylindoles with oxygen is well known (B. Witkop and J. B. Patrick, *J. Am. Chem. Soc.*, **73**, 2196 (1951), and previous papers)].

(15) An attempt to develop the 9-carbinol carbamate grouping from the aldehyde group in VII prior to quinone elaboration failed in the preparation of the intermediate alcohol because of diindolylmethane formation (*cf.* ref. 10 and 13). We interpret the successful preparation of XIII to be the result of significant intervention of structures such as i in the resonance hybrid of the pyrrolo[1,2-*a*]indoloquinone system, which mitigates against the normal electronic effects of the indole system (ii, arrows) present in the carbinol derived from VII.



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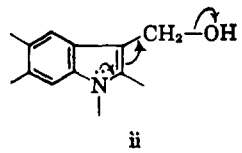

ii

(16) *Cf.* W. M. McLamore, S. Y. P'An, and A. Bavley, *J. Org. Chem.*, **20**, 1379 (1955).