tion; it consists of a nucleation act followed by growth, which is apparently controlled by processes occurring at the particle–solution interface.[5] The endothermic nature of collagen fibrogenesis, as well as tobacco mosaic virus agglomeration, precipitation of denatured proteins, and formation of the mitotic spindle, indicates a fundamental role for hydrophobic bonding.[6] These processes may all involve the expulsion of hydrate water producing the entropy rise necessary to drive the reaction forward.[7] There is, in addition, evidence that fiber growth entails lateral alignment of bonding regions in neighboring molecules by electrostatic and hydrogen bonds.[8–10] Over longer periods, covalent cross-linking leads to the increasingly insoluble network characteristic of mature and aged fibers.

The least studied aspect of the fibrogenesis process is the formation of hydrophobic bonds accompanying hydrate water expulsion. It may be here that unsaturated fatty acids exert their effects.

Acknowledgment.—We thank Miss Catherine Parrott and Mrs. Janet Hoferkamp for their valuable technical assistance.

(5) J. M. Cassel, L. Mandelkern, and D. E. Roberts, *J. Am. Leather Chemists' Assoc.*, **57**, 556 (1962).
(6) W. Kauzmann, *Advan. Protein Chem.*, **14**, 1 (1959).
(7) H. S. Frank, *Ann. N. Y. Acad. Sci.*, **125**, 739 (1965).
(8) H. B. Bensusan and B. L. Hoyt, *J. Am. Chem. Soc.*, **80**, 719 (1958).
(9) G. C. Wood and M. K. Keech, *Biochem. J.*, **75**, 588 (1960).
(10) R. A. Grant, R. W. Horne, and R. W. Cox, *Nature*, **207**, 822 (1965).

The Mitomycin Antibiotics. Synthetic Studies. XXI.[1] Indoloquinone Analogs with Further Variations at C-5

WILLIAM A. REMERS AND MARTIN J. WEISS

Process and Preparations Research Section, Lederle Laboratories Division, American Cyanamid Company, Pearl River, New York

Received March 13, 1968

Indoloquinone carbamate analogs of the mitomycin antibiotics, bearing substituents such as chloro, alkoxy, hydroxy, methylthio, and various amines at the 5 position, were prepared and tested against bacteria. Following the observation of interesting *in vivo* activity for a 5-ethylenimino derivative, analogs containing this group and embodying variants at N-1 and the carbamate nitrogen were also prepared. The *in vitro* activities of the more interesting analogs are reported.

Following the demonstration of interesting antibacterial activity for indoloquinone analogs (*e.g.*, I)[2] of the mitomycin antibiotics, a systematic program of structural variation based on these lead compounds was undertaken. This program has included variants at each position in the pyrrole ring[1,3] and also in the quinone ring.[4]

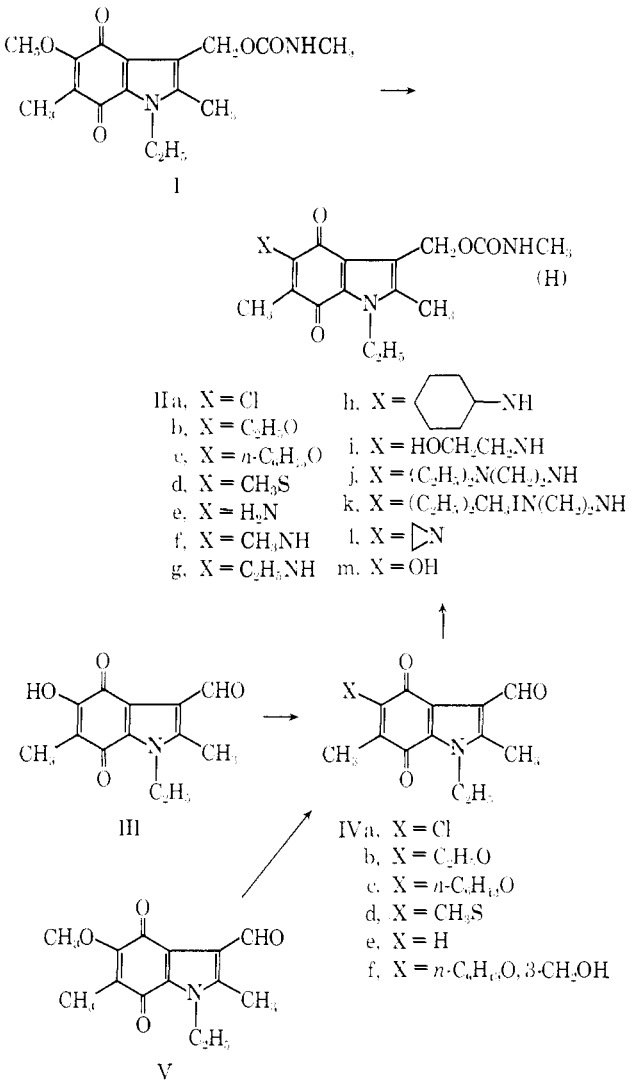
In the present paper we describe the preparation of analogs of I with further variations at position 5 in the quinone ring. This position is of particular importance since at least two substituents, amino and methoxy, at the corresponding position in the mitomycins confer activity.[5] Furthermore, this position has been suggested as a possible active site in the cross-linking of DNA by the mitomycins.[6]

For the preparation of the 5-chloro analog (IIa), 2,6-dimethyl-1-ethyl-5-hydroxy-4,7-indoloquinone-3-carboxaldehyde[2] (III) was warmed with 2 equiv of $POCl_3$ in dimethylformamide,[7] and the resulting 5-chloro-3-carboxaldehyde (IVa) was converted into IIa by the usual technique[2] (Scheme I). Alkoxy derivatives were prepared by treatment of 5-hydroxyindoloquinone-3-carboxaldehyde (III) with ethyl orthocarbonate or with *n*-hexyl orthocarbonate,[8] followed by conversion of the resulting 5-ethoxy (IVb)[9] and 5-*n*-hexyloxy (IVc) derivatives into hydroxymethyl carbamates IIb and IIc in the usual way.[2] Attempts to prepare 5-methylthio analog IId by a route involving displacement of the 5-methoxy group of V with methyl mercaptide ion were unsuccessful. However, treatment of V with methyl mercaptan and HCl afforded 5-methylthioindoloquinone-3-carboxaldehyde (IVd) in low yield, and conversion[2] of IVd into IId proceeded without difficulty. Several attempts to add methyl mercaptan to the 5-unsubstituted indoloquinone-3-carboxaldehyde (IVe)[4b] gave products of indefinite composition.

Treatment of the lead 5-methoxyindoloquinone carbamate (I) with a variety of amines in methanol afforded an interesting series of 5-amino analogs (IIe–l). These reactions were followed by thin layer chromatography (tlc) and were conducted until there was no evidence of starting material. In this manner a rough qualitative estimate of relative reaction rates for the displacement of the methoxy group of I by the various amines was obtained. The primary amines were most reactive, followed in decreasing order of reactivity by ethyleni-

(1) Preceding paper in this series: J. F. Poletto, G. R. Allen, Jr., and M. J. Weiss, *J. Med. Chem.*, **11**, 882 (1968).
(2) G. R. Allen, Jr., J. F. Poletto, and M. J. Weiss, *J. Am. Chem. Soc.*, **86**, 3878 (1964); G. R. Allen, Jr., and M. J. Weiss, *J. Med. Chem.*, **10**, 1 (1967).
(3) (a) G. R. Allen, Jr., L. M. Binovi, and M. J. Weiss, *ibid.*, **10**, 7 (1967); (b) G. R. Allen, Jr., J. F. Poletto, and M. J. Weiss, *ibid.*, **10**, 14 (1967); (c) G. R. Allen, Jr., and M. J. Weiss, *ibid.*, **10**, 23 (1967); (d) J. F. Poletto, G. R. Allen, Jr., and M. J. Weiss, *ibid.*, **10**, 95 (1967).
(4) (a) W. A. Remers and M. J. Weiss, *J. Am. Chem. Soc.*, **88**, 804 (1966); (b) R. H. Roth, W. A. Remers, and M. J. Weiss, *J. Org. Chem.*, **31**, 1012 (1966).
(5) 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).
(6) W. Szybalski and V. N. Iyer, *Fed. Proc.*, **23**, 946 (1964).
(7) This method was developed for hydroxynaphthoquinones by G. R. Allen, Jr. Although the yield was low in the present case, no satisfactory alternate procedure could be found. Reagents such as HCl and $SOCl_2$ caused extensive decomposition of I.
(8) Kindly furnished by R. W. Broschard. The use of orthocarbonates in the preparation of alkylaryl ethers was first reported by B. Smith [*Acta Chem. Scand.*, **10**, 1006 (1956)]. The extension of this method to hydroxyquinones is due to J. B. Patrick, D. B. Cosulich, and R. W. Broschard (private communication).
(9) This experiment was performed by R. H. Roth.

SCHEME I



mine and ammonia. Piperidine (a secondary amine) and aniline did not react with I under these conditions. The reaction of I with ammonia did not go to completion, but the product could be isolated by partition chromatography. Dimethylamine did react with I, but it was not possible to obtain an analytically pure product. However, the disappearance of starting material and appearance of a purple aminoquinone was readily followed by tlc.

Diethylaminoethylamino derivative IIj was useful for the preparation of additional analogs. Thus, it gave with methyl iodide the quaternary salt IIk, and upon acid hydrolysis it afforded 5-hydroxy analog IIm. Preparation of IIm by alkaline hydrolysis of I has been described previously;[4a] however, this method is unreliable and is sometimes complicated by concomitant carbamate hydrolysis. The present method is superior because the water-soluble hydrochloride is converted into the insoluble hydroxy compound (IIm), which precipitates and thus becomes less susceptible to carbamate hydrolysis.

In Table I the uv spectra of a variety of 5-substituted 6-methylindoloquinones in methanol are presented. The complexity of these spectra makes detailed analysis difficult, although several obvious features are of interest. The effect of substituents on the longest wavelength band ($\pi \rightarrow \pi^*$ transition)[10] is pronounced, affording a wide spectrum of visible colors. Another noteworthy feature is that the longest wavelength maxima of the ethylenimino derivative (III) is at a shorter wavelength than that observed with other amines. Apparently the lone pair of this substituent is less able to conjugate with the quinone system than are the lone pairs of other amino groups.[11]

The various methylcarbamates described above are listed in Table II. Several unsubstituted carbamates prepared in the same manner from the corresponding methoxyquinones[4a] are also included in this table. When these compounds were tested *in vivo* against a variety of bacteria it was found that, whereas in general they were less impressive than lead compound I, the 5-ethylenimino analog III was considerably more interesting, particularly with respect to oral activity against certain tetracycline-resistant *Staphylococcus* and *Streptococcus* species. The *in vivo* activity of these compounds is described in detail in an accompanying paper.[12] Following this observation of superior activity for this ethylenimino derivative, we undertook the preparation of a variety of compounds which possessed the 5-ethylenimino group and embodied variations in the substituent on the indole nitrogen, and in the substituent on the carbamate nitrogen. These compounds were obtained by treatment of the corresponding 5-methoxyindoloquinone carbamates[1,3a,c] with ethylenimine in methanol and are listed in Table III. *In vivo* testing of these compounds revealed that the most highly active analog, surpassing the ethylenimine III, was the hydroxyethyl carbamate VId. Several variants of VId were then prepared. In these variants the ethylenimine group was replaced by the 2-methylethylenimine and cyclohexenimine groups (Table III); however, since their activity fell below that of VId, no further substituted aziridines were prepared.

It has been noted that the oral activity of the mitomycins is surprising in view of their susceptibility to acid degradation.[13] Since 5-ethylenimino-substituted indoloquinone analogs of the mitomycins, such as III, also possess significant oral activity it was of interest to determine the case of their hydrolysis and to identify the products of hydrolysis. Treatment of III with aqueous methanol 0.01 *N* in HCl for 10 min, followed by quenching with excess $NaHCO_3$ and extraction with ether, gave a purple solid which showed three spots on tlc. One of these spots was characteristic of 5-hydroxyethylamino derivative IIi, the product of simple hydrolysis of the ethylenimine group. The other two spots could not be identified. The absence of starting material indicated the rapid completion of hydrolysis under these conditions. Solutions of ethyleniminoquinone III in water containing small amounts of dimethyl sulfoxide appear to be stable at room temperature for at least 1 week; however, if the concen-

(10) This transition shifts to shorter wavelength on going from MeOH to the less polar $CHCl_3$.

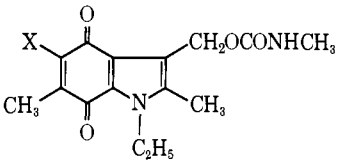
(11) H. C. Brown and A. Tsukamoto [*J. Am. Chem. Soc.*, **83**, 2016 (1961)] have noted that ir absorption spectra of 1-acylaziridines show carbonyl stretching frequency at 1730 cm^{-1}, suggesting little conjugation within the amide group as *contrasted* with the usual tertiary amide.

(12) M. J. Weiss, G. S. Redin, G. R. Allen, Jr., A. C. Dornbush, H. L. Lindsay, J. F. Poletto, W. A. Remers, R. H. Roth, and A. E. Sloboda, *J. Med. Chem.*, **11**, 742 (1968).

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

TABLE I

ULTRAVIOLET ABSORPTION SPECTRA OF INDOLOQUINONES IN METHANOL

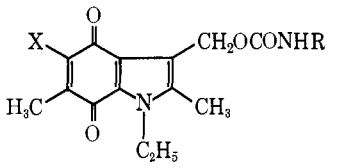


5-Substituent (X)	Ultraviolet and visible maxima, mμ (ε)				Color
H[a]	228 (14,300)	264 (13,600), 273 (11,600)	346 (3500)	441 (2000)	Yellow
CH_3[a]	228 (16,900)	271 (16,500), 278 (16,400)	342 (13,000)	445 (2100)	Orange
C_2H_5O (IIb)	232 (17,700)	286 (14,300)	345 (3500)	460 (1200)	Orange
Cl (IIa)	228 (18,600)	278 (16,300), 284 (16,400)	352 (2540)	463 (1570)	Yellow
HO (IIm)	233 (19,100)	295 (19,000)	345 (4300)	485 (610)	Orange
CH_3S (IId)	236 (17,600)	271 (7800)	348 (5850)	485 (1600)	Orange
▷N (III)	235 (20,700)	308 (14,300)	350 (4300)	485 (1300)	Red
H_2N (IIe)	243 (15,800)	309 (11,000)	348 (4370)	538 (870)	Purple
C_2H_5NH (IIg)[b]	249 (17,300)	316 (12,000)	350 (6000)	550 (1530)	Purple

[a] The preparation of this compound is described in ref 4b. [b] Other primary amines gave spectra nearly identidal with IIg. The dimethylamino derivative could not be obtained pure; however, the impure material showed maxima at 248, 312, 350, and 540 mμ.

TABLE II

C-5 VARIANTS OF 2,6-DIMETHYL-1-ETHYL-3-HYDROXYMETHYL-4,7-INDOLOQUINONE CARBAMATE



C-5 substituent (X)	R	Yield, %	Mp, °C[b]	Formula	Analyses
Cl (IIa)	CH_3	16	182–183	$C_{15}H_{17}ClN_2O_4$	C, H, Cl
C_2H_5O (IIb)	CH_3	35	156–159	$C_{17}H_{22}N_2O_5$	C, H, N
n-$C_6H_{13}O$ (IIc)	CH_3	Low	95.5–96.5	$C_{21}H_{30}N_2O_5$	C, H, N
CH_3S (IId)	CH_3	Low	184–185	$C_{16}H_{20}N_2O_4S$	C, H, S
H_2N (IIe)	CH_3	83	dec 170	$C_{15}H_{19}N_3O_4$	C, H, N
CH_3NH (IIf)[a]	CH_3	79	213–215	$C_{16}H_{21}N_3O_4$	C, H, N
C_2H_5NH (IIg)	CH_3	73	144–148	$C_{17}H_{23}N_3O_4$	C, H
⬡NH (IIh)	CH_3	61	115–117	$C_{21}H_{29}N_3O_4$	C, H, N
$HOCH_2CH_2NH$ (IIi)	CH_3	65	158–160	$C_{17}H_{23}N_3O_5$	C, H, N
$(C_2H_5)_2NCH_2CH_2NH$ (IIj)	CH_3	94	153–155	$C_{21}H_{32}N_4O_4$	C, H, N
$(C_2H_5)_2CH_3INCH_2CH_2NH$ (IIk)[c]	CH_3	79	dec 103	$C_{22}H_{35}IN_4O_4$	C, H, I
▷N (III)	CH_3	72	168–169	$C_{17}H_{21}N_3O_4$	C, H, N
H_2N (IIn)	H	32	200–205	$C_{14}H_{17}N_3O_4$	C, H, N
CH_3NH (IIo)	H	78	236–240	$C_{15}H_{19}N_3O_4$	C, H, N

[a] Prepared by J. F. Poletto from methylamine and 1-ethyl-3-hydroxymethyl-5-methoxy-2,6-dimethylindole-4,7-dione phenylcarbonate.[2] [b] All compounds were recrystallized from CH_2Cl_2–hexane. [c] Prepared by shaking IIj with excess MeI for 3 days.

tration of dimethyl sulfoxide is increased to 40% a slow hydrolysis of the ethylenimine group ensues and tlc shows the formation of 5-hydroxyethylamino derivative IIi, which is considerably less active than III (Table IV). Work-up of the solution after 2 days afforded low yields of both IIi and III.

Biology.—The *in vitro* antibacterial activity[14] of the more interesting indoloquinones having variants at the 5 position, together with the 5-ethyleniminoindoloquinones having variants at N-1 and on the carbamate nitrogen, is given in Table IV. In no compound is the *in vitro* activity significantly improved over that of the lead 5-methoxyindoloquinone I[2] or certain other analogs previously prepared (especially the C-2 variants[3b]). However, several of the 5-ethyleniminoquinones, particularly those with methyl and with hydroxyethyl substituents on the carbamate nitrogen, show oral activity against a variety of gram-positive organisms including resistant *Staphylococcus* and *Streptococcus* strains. This *in vivo* activity is listed and discussed in an accompanying paper.[12]

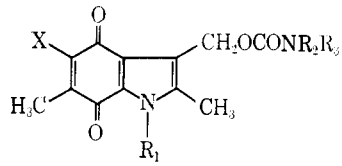
(14) For a complete description of this test procedure as conducted in these laboratories, see G. R. Allen, Jr., B. R. Baker, A. C. Dornbush, J. P. Joseph, H. M. Kissman, and M. J. Weiss, *J. Med. Pharm. Chem.*, **2**, 391 (1960).

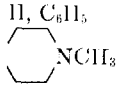
Experimental Section

General.—Melting points were determined on a Mel-Temp melting point apparatus and are corrected. Uv spectra were determined in MeOH on a Cary recording spectrophotometer, and ir spectra in KBr with a Model 21 Perkin-Elmer spectrophotometer. Solutions were dried ($MgSO_4$) and concentrated under reduced pressure on a rotary evaporator. Where analyses

TABLE III

2,6-DIMETHYL-1-ALKYL-5-ETHYLENIMINO-3-HYDROXYMETHYL-4,7-INDOLOQUINONE CARBAMATES



Compd	X	R1	R2, R3	Yield, %	Mp, °C	Formula	Analyses
VIa	▷N	C_2H_5	H, H	64	220–223[a]	$C_{16}H_{19}N_3O_4$	C, H, N
b	▷N	C_2H_5	H, C_2H_5	88	149–152[b]	$C_{18}H_{23}N_3O_4 \cdot 0.5H_2O$	C, H, N
c	▷N	C_2H_5	H, n-C_3H_7	87	152–153[a]	$C_{19}H_{25}N_3O_4$	C, H, N
d	▷N	C_2H_5	H, CH_2CH_2OH	82	136–138[b]	$C_{18}H_{23}N_3O_5$	C, H, N
e	▷N	C_2H_5	H, C_6H_5	74	165–168[a]	$C_{22}H_{23}N_3O_4$	C, H, N
f	▷N	C_2H_5		72	148–151[a]	$C_{21}H_{25}N_4O_4$	C, H
g	▷N	CH_3	CH_3, H	98	196–198[a]	$C_{16}H_{19}N_3O_4$	C, H, N
h	▷N	n-C_3H_7	CH_3, H	81	128–131[a]	$C_{18}H_{23}N_3O_4$	C, H, N
i	▷N	i-C_3H_7	CH_3, H	97	180–181[a]	$C_{18}H_{23}N_3O_4$	C, H, N
j	▷N	CH_2CH_2F	CH_3, H	89	182–185[a]	$C_{17}H_{20}FN_3O_4$	C, H, N, F
k		C_2H_5	CH_2CH_2OH, H	81	142–145[a]	$C_{18}H_{25}N_3O_5$	C, H, N
l		C_2H_5	CH_2CH_2OH, H	91	162–163[b]	$C_{22}H_{29}N_3O_5$	C, H, N

[a] Recrystallized from CH_2Cl_2–hexane. [b] Recrystallized from ether–hexane.

are indicated only by symbols of the elements, analytical results obtained for those elements were within ±0.4% of the theoretical values.

5-Chloro-2,6-dimethyl-1-ethyl-4,7-dioxo-3-indolecarboxaldehyde (IVa).—To an ice-cooled mixture of 1.22 g (8 mmoles) of $POCl_3$ and 4.0 ml of DMF was added a solution of 988 mg (4 mmoles) of 2,6-dimethyl-1-ethyl-5-hydroxy-4,7-indoloquinone-3-carboxaldehyde (V)[2] in 12 ml of DMF. The mixture was heated on a steam bath for 30 min, cooled, poured onto ice, and treated with CH_2Cl_2 and 5% $NaHCO_3$. The organic layer was washed with 5% $NaHCO_3$ until the wash remained colorless, dried, and concentrated and the residue was purified by adsorption chromatography on a magnesia–silica gel column (24 times 120 mm) with CH_2Cl_2 containing 10% of acetone as eluent. Concentration of the orange eluate afforded 90 mg (8.4%) of orange crystals: mp 145–150°; λ_{max} 6.1, 6.3 μ; λ_{max} 236 mμ (ε 15,000), 267 (16,400), 275 (17,500), 287 (16,300), 335 (5850), 437 (2600). *Anal.* ($C_{13}H_{12}ClNO_3$): H, Cl, N; C: calcd, 58.76; found, 59.34.

2,6-Dimethyl-5-ethoxy-1-ethyl-4,7-dioxo-3-indolecarboxaldehyde (IVb).[7]—A mixture of 1.0 g of 2,6-dimethyl-1-ethyl-5-hydroxy-4,7-indoloquinone-3-carboxaldehyde (III)[2] and 20 ml of tetraethyl orthocarbonate[6] was heated at reflux temperature for 3 hr. The excess orthocarbonate was removed by distillation and the residue was treated with EtOH. Recrystallization from EtOH of the solid that formed afforded 880 mg (80%) of dark red crystals: mp 117–119°; λ_{max} 5.98, 6.04, 6.10 μ; λ_{max} 215 mμ (ε 26,000) 245 (7000), 270 (2900), 331 (1700). *Anal.* ($C_{15}H_{17}NO_4$) C, H, N.

2,6-Dimethyl-1-ethyl-5-*n*-hexyloxy-3-hydroxymethylindole-4,7-dione (IVf).—A mixture of 2.0 g of III,[2] 7.5 ml of tetra-*n*-hexyl orthocarbonate,[8] and 20 ml of xylene was heated at reflux temperature for 2 hr, cooled, diluted with ether, and washed twice with $NaHCO_3$. The organic layer was dried and concentrated and the residue was purified by adsorption chromatography on a magnesia–silica gel column with CH_2Cl_2 containing 2% of acetone as eluent. Concentration of eluate from the large orange band that formed afforded 2.04 g of 2,6-dimethyl-1-ethyl-5-*n*-hexyloxy-4,7-dioxo-3-indolecarboxaldehyde (IVc) as an orange oil: λ_{max} 5.9, 6.05, 6.10 μ. Without further purification, this oil was converted directly to the corresponding 3-hydroxymethyl derivative.

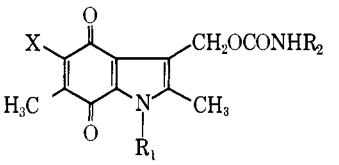
A solution of 2.0 g (6.3 mmoles) of IVc in 560 ml of MeOH, under N_2, was heated to boiling and treated with 2.0 g (excess) of $NaBH_4$ in 40 ml of EtOH. The mixture was stirred at room temperature for 1 hr and treated with 10 ml of acetone. After 5 min, 20 ml of a solution of 1 *N* $FeCl_3$ and 0.1 *N* HCl was added and the resulting mixture was treated with H_2O and CH_2Cl_2. The organic layer was washed ($NaHCO_3$), dried, and concentrated and the residual oil was purified by partition chromatography on 200 g of diatomaceous earth, utilizing a MeOH–heptane system. Concentration of eluate from the principal band (orange) gave a crystalline solid, which on recrystallization from ether–hexane afforded 1.20 g (45% yield from III) of orange needles: mp 70–71°; λ_{max} 2.83, 6.03, 6.09 μ; λ_{max} 232 mμ (ε 16,600), 289 (13,000), 355 (3220), 467 (1290). *Anal.* ($C_{19}H_{27}NO_4$) C, H, N.

2,6-Dimethyl-1-ethyl-5-methylthio-4,7-dioxo-3-indolecarboxaldehyde (IVd).—A solution of 870 mg of 2,6-dimethyl-1-ethyl-5-methoxy-4,7-dioxo-3-indolecarboxaldehyde (V)[2] in 25 ml of AcOH was treated with 1.0 ml of concentrated HCl and 2.0 ml of methyl mercaptan. After 2 days the mixture was poured into a large volume of H_2O and extracted with CH_2Cl_2. This extract was washed (H_2O, $NaHCO_3$), dried, and concentrated. The residue was dissolved in MeOH, treated with excess $FeCl_3$, diluted (H_2O), and extracted (CH_2Cl_2). The red oil obtained on concentration of this extract was dissolved in 25 ml of the upper and 37.5 ml of the lower phase of the system MeOH–heptane, mixed with 50 g of diatomaceous earth and packed atop a column prepared from 375 ml of the lower phase and 500 g of diatomaceous earth. Elution of this column with the upper phase gave in hold-back volumes 2.3–3.1 (750 ml/hbv), after concentration and recrystallization from hexane, 33 mg (3.7%) of orange rods: mp 126–128°; λ_{max} 6.02, 6.17 μ; λ_{max} 268 mμ (ε 17,000), 338 (8000), 475 (2400). *Anal.* ($C_{14}H_{14}NO_3S$) C, H, S.

Preparation of indoloquinone methylcarbamates IIa was carried out by the usual technique.[2] Solutions of the corresponding 3-carboxaldehydes (IVa–d) in MeOH were reduced with $NaBH_4$ to the 3-hydroxyethyl derivatives as described in detail in the preceding example (the *n*-hexyloxy compound). With the exception of this example, these 3-hydroxymethyl compounds were converted directly into the methylcarbamates. For these conversions solutions of the compounds in excess methyl isocyanate were heated at reflux temperature for 20 hr. The excess isocyanate was then removed under reduced pressure and the residual solids were recrystallized from suitable solvents. Table II lists the methylcarbamates IIa–d thus prepared. Their uv spectra are recorded in Table I.

TABLE IV

In Vitro ANTIBACTERIAL ACTIVITY OF THE 2,6-DIMETHYL-3-HYDROXYMETHYLINDOLE-4,7-DIONE CARBAMATES



				Min inhib concn (μg/ml)[a] against					
Compd	X	R_1	R_2	*Staph.* Smith	*Staph.* Rose	*Strep.* C203	*Strep.* β80	*Past.* 310	*Kleb.* 53
	H[4a]	C_2H_5	CH_3	0.8	1.6	3	3		12.5
	CH_3[4b]	C_2H_5	CH_3	1.6	6	6	12.5		
IIa	Cl	C_2H_5	CH_3	6	12.5	6	25	6	25
IIb	OC_2H_5	C_2H_5	CH_3	12.5	25	12.5	50		
IId	SCH_3	C_2H_5	CH_3	6	25	25		6	
IIn	NH_2	C_2H_5	H	12.5	25	25			
IIo	CH_3NH	C_2H_5	H	25	25	6		25	
IIg	C_2H_5NH	C_2H_5	CH_3	3	12.5	25	25		
IIj	$(C_2H_5)_2NCH_2CH_2NH$	C_2H_5	CH_3	25	100	25			
VIa		C_2H_5	H	1.6	3	1.6	6	12.5	
IIm		C_2H_5	CH_3	3	12.5	6	25		
VIc		C_2H_5	n-C_3H_7	6	12	3	6		
VId		C_2H_5	CH_2CH_2OH	1.6	1.6	0.8	12.5		
VIe		C_2H_5	C_6H_5	50	50	25	50		
VIf		CH_3	no H,	6	12.5	3	12.5		
VIg		CH_3	CH_3	1.6	3	0.8	12.5		100
VIh		n-C_3H_7	CH_3	3	6	1.6	12.5		
VIi		i-C_3H_7	CH_3	25	25	25	100		
VIj		CH_2CH_2F	CH_3	1.6	3	0.8	6		
VIk		C_2H_5	CH_2CH_2OH	3	6	3	50		
VIl		C_2H_5	CH_2CH_2OH	12.5	25	6	12.5		

[a] Highest test level: 100 μg/ml. All data are from concurrent assays. Abbreviations for microorganisms: *Staph.* Smith = *Staphylococcus aureus* strain Smith, ATCC 13709; *Staph.* Rose = *S. aureus* strain Rose, ATCC 14154; *Strep.* C203 = *Streptococcus pyogenes*, C203; *Strep.* β80 = *Streptococcus* sp., β-hemolytic 80; *Past.* 310 = *Pasteurella multocida*, ATCC 310; *Kleb.* AD = *Klebsiella pneumoniae*, AD.

Preparation of Carbamates With Various Amines at C-5 (IIa–i, III, and VIa–l).—The appropriate 5-methoxyindoloquinone carbamate was dissolved in MeOH and treated with an excess of the amine. In most exampes 100 mg of the quinone, 15 ml of MeOH, and 0.5 ml of amine were used. For gaseous amines the MeOH was saturated with the amine. The progress of the reaction was followed by tlc with the upper phase of a Me_2CO–C_6H_6–H_2O (2:1:2) system as solvent, and the purple or wine color of the amino derivative could be easily distinguished from the yellow starting material. From these observations qualitative estimates of relative reaction rates were made. With primary amines the reactions were completed in 16 hr. Ethylenimine required 3 days for complete reaction, and NH_3 did not give a complete reaction in 9 days. Piperidine and aniline gave no reaction under these conditions. After completion of the reactions, the solutions were concentrated under reduced pressure and the residual solids were recrystallized from appropriate solvents. In the case of the 5-amino analog with an unsubstituted carbamate (IIn) the crude product could not be purified by recrystallization until it was first resolved by partition chromatography on diatomaceous earth with the same system as described in the preparation of the 5-*n*-hexyloxy-3-hydroxymethylindoloquinone. The various 5-amino analogs are listed in Tables II and III. Typical uv spectra are reported in Table I.

2,6-Dimethyl-1-ethyl-5-hydroxy-3-hydroxymethylindole-4,7-dione (IIm).—A suspension of 425 mg of 5-[2-(diethylamino)-ethylamino]-2,6-dimethyl-1-ethyl-3-hydroxymethylindole-4,7-dione methylcarbamate (IIi) in 175 ml of H_2O was treated with 27 drops of 3 *N* HCl. The purple solution that formed immediately gradually turned pink and a precipitate formed. After 1 hr excess Na_2CO_3 was added and the blue solution was extracted with CH_2Cl_2. The aqueous layer was carefully acidified with HCl until it turned orange and was then extracted (CH_2Cl_2). This extract was dried and then concentrated as hexane was added. When the first crystals appeared the solution was cooled. This procedure gave 160 mg (50%) of orange crystals identical in melting point, mixture melting point, and ir spectrum with IIn prepared from alkaline hydrolysis of the corresponding 5-methoxyquinone methylcarbamate.[4a]

Hydrolysis of 2,6-Dimethyl-1-ethyl-5-ethylenimino-3-hydroxymethylindole-4,7-dione Methylcarbamate. A. With 0.01 *N* HCl.—A solution of 12 mg of 2,6-dimethyl-1-ethyl-5-ethylenimino-3-hydroxymethylindole-4,7-dione methylcarbamate (III) in 3 ml of MeOH was diluted with 6 ml of H_2O and treated with 0.03 ml of 3 *N* HCl. After 10 min the resulting purple solution was neturalized with excess $NaHCO_3$, diluted (H_2O), and extracted (CH_2Cl_2). This extract was dried and concentrated. The residue gave three spots on tlc with Me_2CO–C_6H_6–H_2O (2:1:2) solvent system. One of the spots had an R_f value identical with that of 2,6-dimethyl-1-ethyl-5-(2-hydroxymethylamino)-3-hydroxymethylindole-4,7-dione methylcarbamate (IIi) which was run on the same plate.

B. With Aqueous Dimethyl Sulfoxide.—A solution of 16.5 mg of IIm in 4 ml of DMSO was diluted with 6 ml of H_2O and then kept at 25° for 2 days. The resulting mixture was diluted with 60 ml of H_2O and extracted with CH_2Cl_2. This extract was washed twice with H_2O, dried, and concentrated. The residue was resolved into two components by adsorption chromatography on a magnesia–silica gel column. The component eluted by CH_2Cl_2 was identical in ir spectrum with starting material, and the component eluted by CH_2Cl_2 containing 25% of Me_2CO was identical in ir spectrum with IIi.

Acknowledgment.—We wish to thank Mr. L. M. Brancone and staff for microanalyses, Mr. W. Fulmor and staff for spectral data, Mr. A. C. Dornbush and staff for *in vitro* antibacterial assays, and Dr. G. R. Allen, Jr., for generously furnishing all starting materials. We also thank Miss R. H. Roth and Mr. J. F. Poletto for permission to publish the indicated experiments.

The Mitomycin Antibiotics. Synthetic Studies. XXII.[1] Antibacterial Structure–Activity Relationships in the Indoloquinone Series

MARTIN J. WEISS, GUNNAR S. REDIN, GEORGE R. ALLEN, JR., ALBERT C. DORNBUSH, HARRY L. LINDSAY, JOHN F. POLETTO, WILLIAM A. REMERS, RETA H. ROTH, AND ADOLF E. SLOBODA

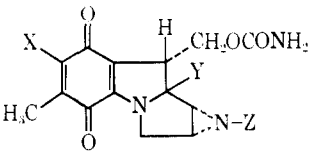
Lederle Laboratories Division, American Cyanamid Company, Pearl River, New York

Received March 13, 1968

The *in vivo* antibacterial structure-activity relationships of a considerable number of indoloquinone analogs of the mitomycin antibiotics are reviewed. The most active members of the series were certain 5-ethyleniminobo derivatives, of which 1-ethyl-2,6-dimethyl-5-ethyleniminoindoloquinone N-(β-hydroxyethyl)carbamate (IV) was the most extensively investigated. This compound shows potent oral activity in mice infected with a spectrum of representative gram-positive organisms, but apparently still retains some of the cytotoxicity manifested by the parent antibiotics and therefore is not of clinical interest.

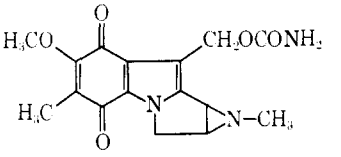
In previous papers of this series we have described the synthesis and *in vitro* antibacterial activity of a series of indoloquinone analogs of the mitomycin antibiotics. Our interest in these antibiotics derives from their very potent broad-spectrum oral antibacterial activity in mice, an activity which extends to tetracycline- and penicillin-resistant strains.[2] Moreover, the mitomycins show an important antitumor effect, and, in fact, mitomycin C has found clinical use in this connection, particularly in Japan.[3] On the other hand, these substances are powerful general cytotoxic agents, which precludes them from consideration as clinical antibacterial agents and presumably limits their utility as antitumor agents. Thus, the mitomycins represent a challenging structure-activity problem, and in this paper we review the relationships which we have found for the indoloquinone series with regard to *in vivo* antibacterial activity.

The structures of the four mitomycin antibiotics were determined by Webb and coworkers[4] and are as shown below.

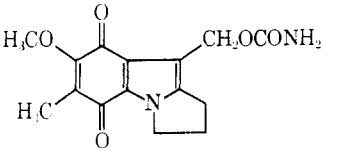


	X	Y	Z
Mitomycin A	OCH_3	OCH_3	H
Mitomycin B	OCH_3	OH	CH_3
Porfiromycin	NH_2	OCH_3	CH_3
Mitomycin C	NH_2	OCH_3	H

The conversion of mitomycin B to the equally potent pyrroloindoloquinone I was achieved by Patrick and coworkers.[5] And in a further simplification the desazairidino analog of I, the pyrroloindoloquinone 7-methoxymitosene (II), was synthesized in our laboratory.[6] Although this latter substance was considerably less potent than the parent antibiotics or I, it retained enough antibacterial effect to be of significant interest. *In vitro* it was markedly active against a variety of gram-positive organisms, including strains resistant to tetracycline and penicillin, but it was relatively ineffective *vs.* gram-negative organisms. In the *Staphylococcus aureus*, strain Smith, infection in mice, II was about one-third as active orally as tetracycline hydro-



I



II

(1) Paper XXI: W. A. Remers and M. J. Weiss, *J. Med. Chem.*, **11**, 737 (1968).

(2) (a) T. Hata, Y. Sano, R. Sugawara, A. Matsumae, K. Kanamori, T. Shima, and T. Hoshi, *J. Antibiotics* (Tokyo), **A9**, 141 (1956); (b) C. L. Stevens, K. G. Taylor, M. E. Munk, W. S. Marshall, K. Noll, G. D. Shah, L. G. Shah, and K. Uzu, *J. Med. Chem.*, **8**, 1 (1965); (c) A. C. Dornbush and G. S. Redin, unpublished data.

(3) R. Jones, Jr., U. Jonsson, J. Colsky, H. E. Lessner, and A. Franzino in "Fourth National Cancer Conference Proceedings, 1960," J. B. Lippincott, Philadelphia, Pa., 1961, p 175; B. Sokoloff, *et al.*, *Growth*, **23**, 109 (1959); L. H. Manheimer and J. Vital, *Cancer*, **19**, 207 (1966); M. Kutsumi, *Wakayama Med. Rept.*, **9**, 153 (1965).

(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, 3187 (1962); see also A. Tulinsky, *ibid.*, **84**, 3188 (1962).

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

(6) G. R. Allen, Jr., J. F. Poletto, and M. J. Weiss, *ibid.*, **86**, 3877 (1964); *J. Org. Chem.*, **30**, 2897 (1965).