related researches on the total synthesis of nonaromatic steroids, which has been provided by Merck and Co., Inc., the Upjohn Co., the U. S. Public Health Service, the National Science Foundation, and the Wisconsin Alumini Research Foundation through the University of Wisconsin Research Committee.

The Mitomycin Antibiotics. Synthetic Studies. XII.[1] Indoloquinone Analogs with Variations at Positions 5 and 6

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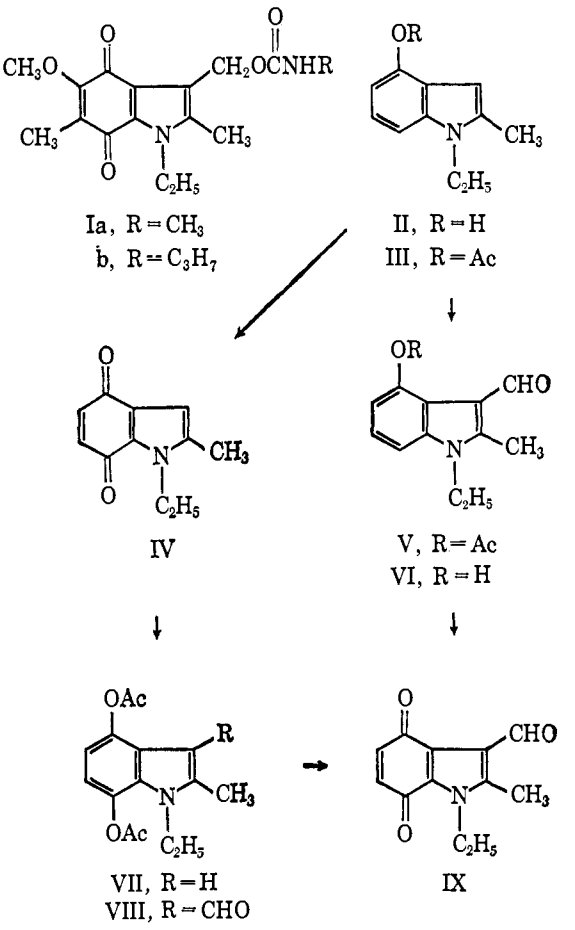
Contribution from the Organic Chemical Research Section, Lederle Laboratories Division, American Cyanamid Company, Pearl River, New York. *Received October 7, 1965*

Abstract: A synthesis of 1-ethyl-3-formyl-2-methyl-4,7-indoloquinone (IX) was devised. Substitution into the quinone ring of IX was effected by use of bromine and of *p*-toluenethiol. The dibromo derivative XI was converted to isomeric bromomethoxyindoloquinones. Treatment of these (separated) isomers with sodium borohydride afforded in each case 3-hydroxymethylindoloquinones that had lost bromine, in addition to the anticipated bromo compounds. The nature of this bromine loss is discussed. When bromomethoxyindoloquinone carbamates were treated with ammonia, the methoxy group was lost in preference to the bromine. Participation of the bromine in accelerating the displacement of methoxy by ammonia is described. Structures of the indoloquinone carbamates derived from the isomeric bromomethoxy compounds and from isomeric *p*-toluenethioindoloquinones were defined by unequivocal synthesis of 1-ethyl-3-hydroxymethyl-6-methoxy-2-methyl-4,7-indoloquinone *n*-propylcarbamate (XVIIb), a compound prepared in each of these series, by a route involving Hooker oxidation of a corresponding 5-hydroxy-6-methylindoloquinone.

Following the demonstration of antibacterial activity for indoloquinone analogs (I)[2a] of the mitomycins,[2b] a comprehensive program of structural variation on these lead compounds was undertaken. In this paper we describe the preparation of analogs of I having a variety of substituents at positions 5 and 6 of the quinone ring, with the remainder of the molecule unchanged. Our approach to these analogs was based on the synthesis of a key indoloquinone (*e.g.*, IX) unsubstituted in the quinone ring, but having the requisite alkyl groups at positions 1 and 2, and at position 3 a formyl group that could be transformed into a hydroxymethylcarbamate[2a] after appropriate substitution into the quinone ring.

The preparation of formylquinone IX from 1-ethyl-4-hydroxy-2-methylindole (II)[3] required two critical operations: (1) introduction of a 3-formyl group and (2) oxidation of a 4-hydroxyindole to the corresponding *p*-quinone. Our first attempt was to deal with these operations in the enumerated order. Direct Vilsmeier–Haack formylation[4] of hydroxyindole II afforded only a very low yield of 3-formyl-4-hydroxyindole VI, with much tar formation. However, by converting II to an acetate, formylating, and deacetyling (II → III → V → VI), an acceptable over-all yield (24%) of VI was obtained. Oxidation of VI to formylquinone IX proved somewhat disappointing. Fremy's salt (potassium nitrosodisulfonate),[5] which had successfully oxidized a 3-formyl-5-hydroxyindole to the corresponding *o*-quinone and a 3-formyl-4-aminoindole to a *p*-quinone,[2a] effected only a partial conversion of VI



(1) Preceding paper in this series: G. R. Allen, Jr., and M. J. Weiss, *Chem. Ind.* (London), submitted for publication.
(2) (a) G. R. Allen, Jr., J. F. Poletto, and M. J. Weiss, *J. Am. Chem. Soc.*, **86**, 3878 (1964); (b) 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, *ibid.*, **84**, 3185 (1962).
(3) W. A. Remers and M. J. Weiss, *ibid.*, **87**, 5262 (1965).
(4) A. Vilsmeier and A. Haack, *Ber.*, **60**, 119 (1927).
(5) See H. J. Teuber and G. Jellinek, *ibid.*, **85**, 95 (1952), and subsequent papers.

to IX, and several recycles were required to raise this conversion to an acceptable level. Furthermore, traces of VI in the quinone product IX were difficult to remove and, if allowed to remain, interfered with subsequent utilization of IX. However, by this method it was possible to obtain enough pure IX for purposes of characterization. Other oxidizing agents, such as ammonium persulfate,[6] were destructive to VI.

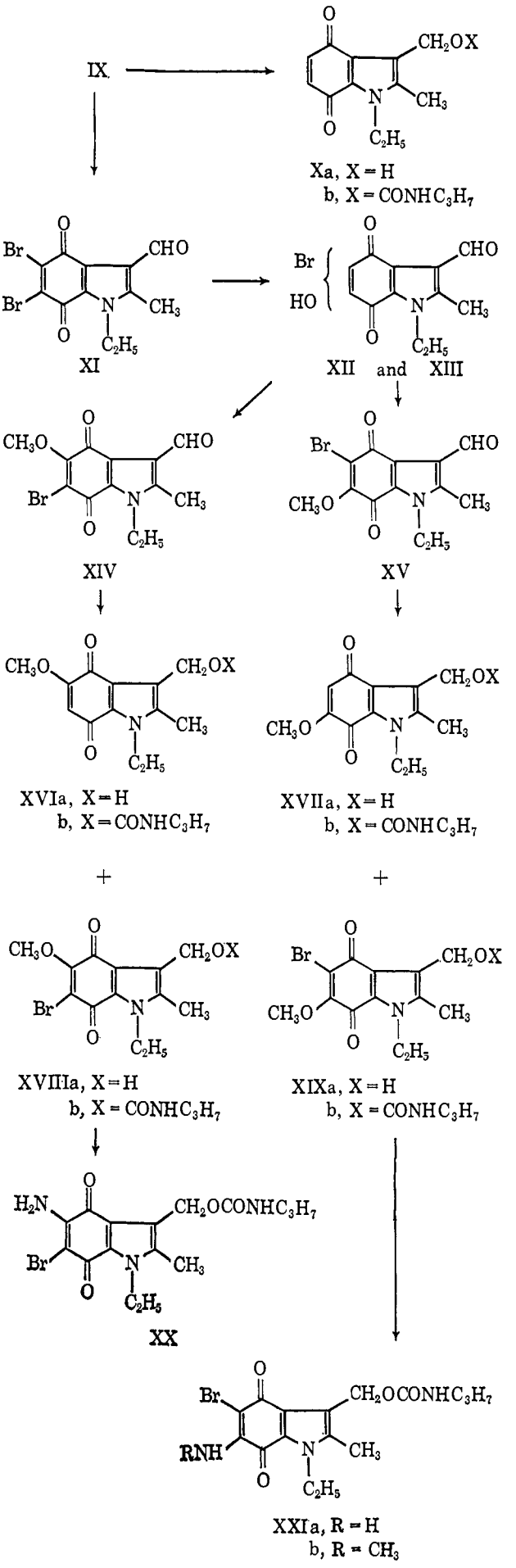
Inversion of the sequence led to a more satisfactory preparation of IX from hydroxyindole II. In this sequence, Fremy's salt oxidation of II to 3-unsubstituted *p*-quinone IV proceeded readily, affording IV in yields up to 68%. A small amount (12%) of purple solid that appeared by nmr spectrum to be an *o*-quinone was also produced. It was not possible to obtain satisfactory combustion analyses for this compound.

The apparent deactivating effect of the 3-formyl group in 4-hydroxyindole VI toward Fremy's salt oxidation is not clearly understood. One possible explanation is that a strong hydrogen bond between the hydroxyl hydrogen and the formyl oxygen[7] renders this hydrogen less available for abstraction by the nitrosodisulfonate radical. Hence, the subsequent steps in the oxidation sequence are inhibited.

Formylation of *p*-quinone IV was accomplished by a sequence involving reductive acetylation,[8] formylation under mild conditions, and alkaline hydrolysis followed by ferric chloride oxidation. The over-all yield for this sequence (IV → VII → VIII → IX) was 44%. Direct formylation of 3-unsubstituted quinone IV was briefly investigated,[9] but abandoned when extensive tar formation occurred.

Formylquinone IX was converted to the corresponding 3-hydroxymethyl derivative Xa by sodium borohydride reduction and ferric chloride oxidation.[2a] Treatment of Xa with *n*-propyl isocyanate[2a] afforded the corresponding carbamate Xb. The yields for both of these reactions were low.

Having thus prepared the 5,6-unsubstituted analog of Ib we now turned our attention to the introduction of various substituents into the quinone ring of formylquinone IX in order to prepare the corresponding 5- and/or 6-substituted analogs of Ib. Chlorination of IX with chlorine was unsuccessful. However, it was possible to prepare crystalline dibromoquinone XI in 25% yield by treating IX with 2 moles of bromine in acetic acid. A small amount of monobrominated quinone also was obtained. Increasing the amount of bromine to 4 moles raised the yield of XI to 83%. Treatment of XI with excess sodium hydroxide in methanol–water followed by acidification afforded a



(6) M. F. Ansell, B. W. Nash, and D. A. Wilson, *J. Chem. Soc.*, 3028 (1963).

(7) The possibility of hydrogen bonding is supported by observations from the infrared absorption spectra wherein the carbonyl stretching frequency of VI is at 6.25 μ, whereas that of the corresponding acetate V is at 6.10 μ, and the OH stretching frequency of VI is at 2.9 μ and of lesser intensity than that at 3.0 μ for nonformylated hydroxyindole II. 5-Hydroxy-3-formylindoles, in which the possibility of intramolecular hydrogen bonding does not exist, are smoothly oxidized by Fremy's salt in high yield to the corresponding *o*-quinones (see ref 2a).

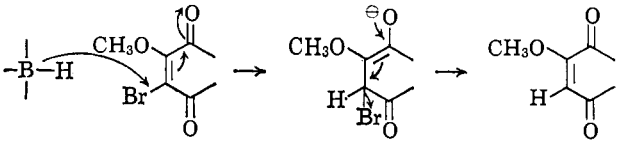
(8) The procedure of L. F. Fieser, W. P. Campbell, E. M. Fry, and M. D. Gates, Jr, *J. Am. Chem. Soc.*, **61**, 3216 (1939), was excellent for this transformation. No formation of a 3-acetylindole was observed.

(9) We have previously noted, W. A. Remers, R. H. Roth, and M. J. Weiss, *ibid.*, **86**, 4612 (1964), that conjugation of a carbonyl group with an indole system deactivates this system toward electrophilic substitution reactions such as the Vilsmeier–Haack formylation.

mixture of isomeric bromohydroxyquinones XII and XIII. This mixture was methylated by diazomethane and the resulting isomeric bromomethoxyquinones XIV and XV were separated by partition chromatography.

Sodium borohydride reduction–ferric chloride oxidation of the isomeric bromomethoxyquinone-3-carboxaldehydes XIV and XV gave surprising results. With each isomer a mixture of at least three products was obtained. Partition chromatography resolved the two mixtures into the desired 3-hydroxymethylbromomethoxyquinones XVIIIa and XIXa, the 3-hydroxymethylmethoxyquinones XVIa and XVIIa lacking bromine (in appreciable yields, as high as 24% for XVIa), and bromine-containing (Beilstein test) quinones that lacked a 3-hydroxymethyl group (infrared evidence). The last-mentioned compounds were not characterized completely, but are possibly diindolylmethanes derived from the hydroquinones of XVIIIa and XIXa.[10,11] In both series the bromo and the debromoquinones having 3-hydroxymethyl groups were converted to the corresponding *n*-propylcarbamate analogs XVIb–XIXb. The synthesis of the 6-methoxyquinone carbamate XVIIb by an independent and unequivocal route, described below, established its structure and hence, the structures of all other compounds derived *via* alkaline hydrolysis of the dibromoquinone XI.

Since the elimination of bromine from bromomethoxyquinones XIV and XV by sodium borohydride in methanol was quite unexpected, we sought to determine the stage (quinone or hydroquinone) at which it was lost. Inasmuch as we were unable to find any examples of bromine loss on treating a bromine-containing electron-rich benzenoid system[12] with sodium borohydride, the most reasonable explanation appeared to involve a loss of bromine at the quinone stage, perhaps *via* hydride addition followed by elimination.



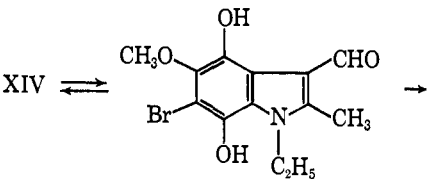
However, as discussed below, the methoxy group of a bromomethoxyquinone undergoes displacement in preference to the bromide when treated with nucleophilic reagents (amines), and this observation cast doubt on the above supposition. That, in fact, debromination had occurred from a bromohydroquinone intermediate (XXII or XXIII) was demonstrated by treatment of bromohydroquinone XXII with sodium borohydride to give, after ferric chloride oxidation, the mixture previously observed. The intermediate bromohydroquinone XXII was obtained from 6-bromo-5-methoxyquinone-3-aldehyde (XIV) on reduction with sodium hydrosulfite;[13] that no bromine loss had occurred during this preparation was shown by the reoxidation of XXII to the starting bromoquinone XIV in almost quantitative yield.

(10) Diindolylmethane formation from 3-hydroxymethylindoles is frequently observed: E. Leete, *J. Am. Chem. Soc.*, **81**, 6023 (1959).

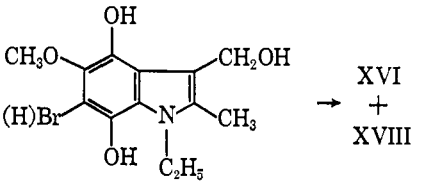
(11) For a discussion of quinone stabilization of the indole 3-hydroxymethyl group, see G. R. Allen, Jr., J. F. Poletto, and M. J. Weiss, *J. Org. Chem.*, 30, 2897 (1965).

(12) However, the loss of chlorine from polynitro chlorobenzenes on treatment with sodium borohydride has been reported by L. A. Kaplan, *J. Am. Chem. Soc.*, **86**, 740 (1964).

(13) L. F. Fieser and M. D. Gates, Jr., *ibid.*, **63**, 2948 (1941).



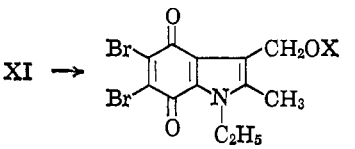
XXII



XXIII

An explanation for this observation might be found in the study of Karabatsos and co-workers[14] on the reductive dehalogenation of aromatic halides with lithium aluminum hydride. This study revealed that the over-all reaction mechanism is nucleophilic substitution and that two structural features enhance the reaction rate. These features are (1) ground-state steric interactions between halogen and neighboring groups and (2) the presence of *ortho* or *peri* groups which can form alkoxides with lithium aluminum hydride. Although in the present examples the reagent is sodium borohydride, both conditions for facilitating dehalogenation are met in XXII. It therefore seems likely that these examples represent another instance of aromatic debromination *via* hydride displacement.

Consistent with the above-noted loss of bromine during sodium borohydride reduction of the bromomethoxyquinones was the observation that bromine was also lost from dibromoquinone XI under similar conditions. Thus propylcarbamate analog XXIVb, prepared from the resulting 3-hydroxymethylquinone XXIVa, gave combustion analysis values that indicated a 16.5% loss of total bromine.



XXIVa, X=H
b, X=CONHC$_3$H$_7$

In an attempt to prepare aminomethoxyquinone[15] analogs of Ib, we treated bromomethoxyquinone carbamates XVIIIb and XIXb with anhydrous ammonia in methanol. Unexpectedly, the resulting incorporation of the amine group was attended in each case with loss of a methoxy group and the product isolated was an aminobromoquinone carbamate (XX from XVIIIb and XXIa from XIXb). Treatment of XIXb with methylamine also eliminated methoxide, and bromomethylaminoquinone XXIb was obtained. A search of the literature disclosed that Marxer had previously noted[16] preferential replacement of the methoxy groups in 2,6-dibromo-3,5-dimethoxybenzoquinone by ethylenimine.

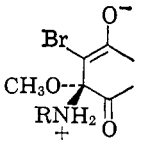
(14) G. J. Karabatsos, R. L. Shone, and S. E. Scheppele, *Tetrahedron Letters*, **31**, 2113 (1964).

(15) An important feature of the antibiotic streptonigrin is the aminomethoxyquinone system: K. V. Rao, K. Biemann, and R. B. Woodward, *J. Am. Chem. Soc.*, **85**, 2532 (1963).

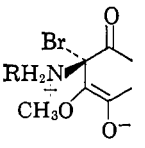
(16) A. Marxer, *Helv. Chim. Acta*, **40**, 502 (1957).

We have noted further that the bromine of a bromomethoxyquinone not only remains on the quinone ring while methoxide is displaced by amines, but that it actually appears to facilitate the displacement of methoxide by these reagents. Thus, the complete displacement by ammonia of methoxide from 5-methoxy-6-bromoquinone carbamate XVIIIb required only several hours, whereas this displacement from the closely related 5-methoxy-6-methylquinone carbamate Ia[2a] was not even complete after 1 week.[17]

These interesting results might be understood in terms of a mutual interaction wherein the bromine substituent *activates* the methoxy group toward nucleophilic displacement while the methoxy group *deactivates* the bromine.[18,19] The relative reactivity of methoxy and the accelerative effect of bromine are based on the comparative energies of the transition states leading to intermediates i and ii, not on the relative aptitude as leaving groups of bromine and methoxy. That the transition state (related to i) leading to displacement of methoxy is the one of lower energy is due to the inductive effect of bromine,[19] which affords greater stabilization to the partial negative charge on neighboring oxygen than would methoxy in the corresponding transition state (related to ii) leading to displacement of bromide.[20]



i



ii

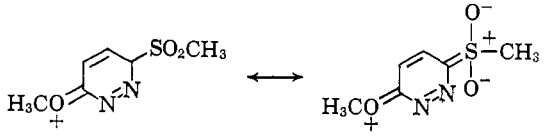
Despite the reported[16] conversion of dibromodiethyleniminobenzoquinones to the corresponding diethyleniminodimethoxybenzoquinones with sodium methoxide, we were unable to prepare by this method an aminomethoxyquinone from aminobromoquinone XXIa, and starting material was recovered.[21]

Although we were able by the fortuitous circumstances described above to prepare the isomeric methoxyquinone carbamate analogs XVIIb and XVIIIb, we had also undertaken a rational synthesis of these analogs, based on the addition of *p*-toluenethiol to 5,6-unsubstituted quinone-3-carboxaldehyde IX. Treatment of IX with 1 mole of *p*-toluenethiol and 1 mole of ferric chloride afforded in 82% yield a mixture of isomeric *p*-toluenethioquinones XXV and XXVII, the 6-substituted quinone XXV (see below) predominating in a ratio of 4:1.[22] Use of excess *p*-toluenethiol gave, in addition to these isomers (isolated by fractional crystallization in a ratio of 6 of XXV to 1 of XXVII), a considerable amount of *bis-p*-toluenethioquinone XXIX. Each of the mono-*p*-toluenethioquinones could be hydrolyzed by dilute sodium hydroxide[23] to afford hydroxyquinones that had indicator properties (yellow in acid, maroon in base).[24] Only the hydroxyquinone XXVI from the major *p*-toluenethioquinone isomer XXV was prepared in sufficient quantity for further characterization. This hydroxyquinone was converted with diazomethane to the corresponding methoxyquinone XXVIII, treatment of which with sodium borohydride in methanol followed by acidic ferric chloride afforded a 3-hydroxymethylquinone identical in infrared spectrum with XVIIa. Brief heating with propyl isocyanate then gave the corresponding carbamate, identical by the usual criteria with XVIIb prepared from methoxybromoquinone XV as described above.

At this point we had several series of compounds with variants at C-5 and C-6, derived from the isomeric methoxybromoquinones and from the isomeric *p*-toluenethioquinones. These series were so interrelated (see flowsheets) that once an unequivocal assignment of structure could be made for any one of the compounds involved, the structures of all the other compounds would follow. An unequivocal synthesis of one of these compounds (XVIIa and derived carbamate XVIIb) was achieved *via* a Hooker oxidation. In this reaction[25] a quinone, bearing on adjacent positions hydroxyl and alkyl groups, is converted to the corresponding quinone bearing hydroxyl and hydrogen or alkyl (shortened by loss of a methylene unit) groups in an inverted arrangement. Thus, a 5-hydroxy-6-methylindoloquinone would be expected to give the corresponding 5-hydrogen-6-hydroxyindoloquinone on Hooker oxidation. In fact, Hooker oxidation of 5-hydroxy-6-methylindoloquinone 3-hydroxymethylcarbamate XXX,[26] prepared[17] from the corresponding

(17) See the Experimental Section.

(18) The methoxy group of 3-methoxy-6-methylsulfonylpyridazine undergoes displacement in preference to the methylsulfonyl group on treatment with sulfanilamide anion; however, in 3,6-dimethoxypyridazine the methoxy groups are unreactive toward this reagent,[19] and furthermore, the methylsulfonyl group is usually highly reactive toward nucleophilic displacement. Shepherd and Fedrick have explained[19] this noteworthy result as a mutual interaction between the substituents, *i.e.*



wherein the methylsulfonyl group is deactivated toward nucleophilic displacement while the methoxy group is activated. This interpretation is pertinent to our observations in the quinone system, since the transition states leading to nucleophilic substitution in quinones closely resemble those in the azine and other aromatic systems.[20]

(19) R. G. Shepherd and J. L. Fedrick, *Advan. Heterocyclic Chem.*, **4**, 199, 222, 230 (1965).

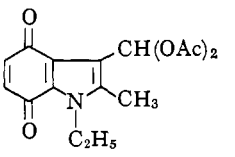
(20) We are grateful to Dr. R. G. Shepherd for a helpful discussion on these aspects of nucleophilic aromatic substitution.

(21) It is to be noted, however, that there is a difference in behavior between ethyleneimino and ordinary amino groups in conjugation with carbonyl groups, the former tending to have less amide nitrogen character than the latter: H. C. Brown and A. Tsukamoto, *J. Am. Chem. Soc.*, **83**, 2016 (1961).

(22) This ratio was indicated by infrared examination of the crude mixture (see the Experimental Section).

(23) Procedure of J. W. MacLeod and R. H. Thomson, *J. Org. Chem.*, **25**, 36 (1960). In this paper the authors provide evidence that when a group is replaced from a naphthoquinone having one unoccupied position in the quinone ring, the entering group assumes the same position that the departing group had occupied.

(24) An attempt to prepare these hydroxyquinones by a route involving Thiele acetoxylation of IX was not successful. With acetic anhydride and a trace of sulfuric acid, the only product obtained from IX was the 3-diacetoxymethyl derivative iii.

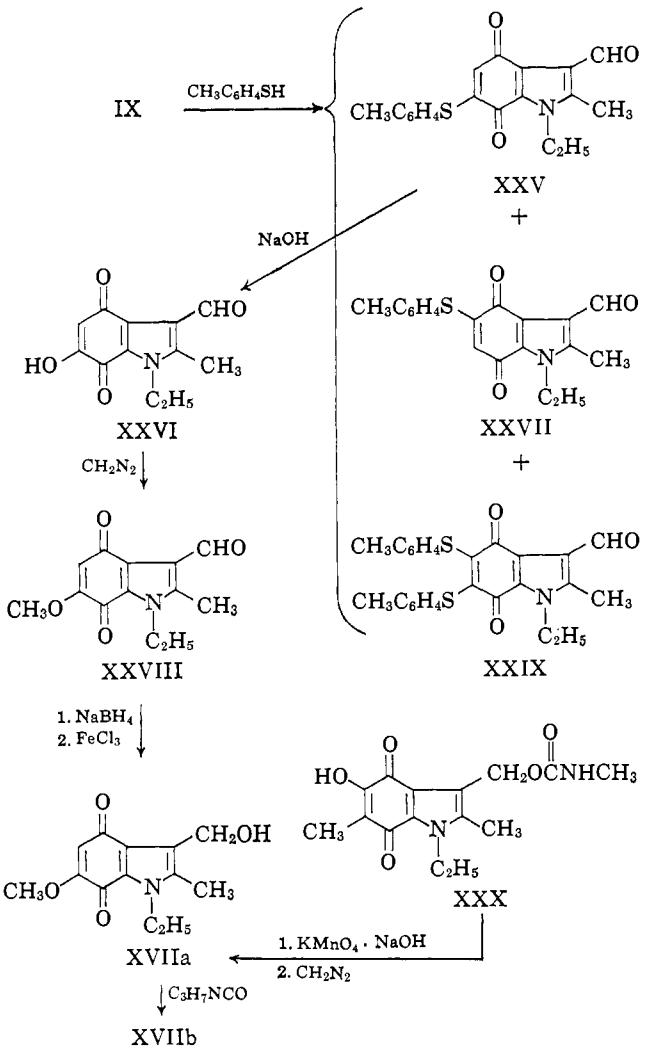


iii

(25) S. C. Hooker, *J. Am. Chem. Soc.*, **58**, 1163, 1174 (1936); S. C. Hooker and A. Steyermark, *ibid.*, **58**, 1179 (1936); L. F. Fieser, J. L. Hartwell, and A. M. Seligman, *ibid.*, **58**, 1223 (1936); L. F. Fieser and M. Fieser, *ibid.*, **70**, 3215 (1948).

(26) A previous attempt to establish identity with XXVI by Hooker oxidation of the corresponding 5-hydroxy-6-methylindoloquinone-3-carboxaldehyde[2a] was unsuccessful. Dr. J. S. Webb of these labora-

5-methoxy-6-methylindoloquinone Ia of unequivocal structure,[2a] followed by diazomethane treatment of the resulting hydroxyquinone, gave 3-hydroxymethyl-6-methoxyindoloquinone XVIIa, identical in infrared spectrum with the samples prepared from XV (bromomethoxyquinone series) and XXV (toluenethioquinone series). Treatment with *n*-propyl isocyanate converted XVIIa from the Hooker oxidation to XVIIb, identical in infrared spectrum and melting point and by mixture melting point comparisons with the samples prepared from XV and XXV. Hence, the structures of all interrelated compounds are established to be those depicted in the flowsheets. *In vivo* assay of the various quinone



carbamates described above against *Staphylococcus aureus* var. Smith[27] indicated that XVIb, the 6-demethyl analog of lead compound Ib, was more active than any of the others, although its level of antibacterial activity was slightly lower than that of Ia or Ib.

Experimental Section

Melting points were determined on a Kofler hot-stage microscope and are corrected. Ultraviolet spectra were determined in methanol solution with a Cary recording spectrophotometer. Infrared spectra were determined in potassium bromide disks with a Perkin-Elmer Model 21 spectrophotometer. Nuclear magnetic resonance spectra were determined in deuteriochloroform, unless otherwise specified, with a Varian A-60 spectrometer. Solutions were dried over anhydrous magnesium sulfate and concentrated under reduced pressure on a rotary evaporator.

4-Acetoxy-1-ethyl-2-methylindole (III). A solution of 4.0 g (22.4 mmoles) of 1-ethyl-4-hydroxy-2-methylindole (II)[3] in 75 ml of water containing 1.35 g (33.3 mmoles) of sodium hydroxide was treated with 3.4 g (33.3 mmoles) of acetic anhydride and 3.0 g (33.3 mmoles) of sodium acetate. After 20 min the mixture was filtered and the solid was dissolved in methylene chloride, washed two times with sodium bicarbonate solution, dried, and concentrated. The dark oily residue was extracted with 40 ml of boiling *n*-hexane. After decantation from an initially formed oil, this extract gave on cooling 2.85 g (58%) of 4-acetoxy-1-ethyl-2-methylindole (III) as white crystals: mp 71–73°; λ_{max} 5.75 μ 223 mμ; (ϵ 31,000), 270 (6750), 287 (5000), 296 (5900). An analytical sample, recrystallized from hexane, had mp 73–74°.

Anal. Calcd for $C_{13}H_{15}NO_2$ (217.26): C, 71.86; H, 6.96; N, 6.45. Found: C, 71.54; H, 7.16; N, 6.20.

4-Acetoxy-1-ethyl-2-methyl-3-indolecarboxaldehyde (V). To an ice-cooled mixture of 2.0 g (13.7 mmoles) of phosphorus oxychloride and 15 ml of dimethylformamide was added dropwise a solution of 3.20 g (13.7 mmoles) of 4-acetoxy-1-ethyl-2-methylindole (III) in 15 ml of dimethylformamide. After 90 min the resulting yellow solution was poured onto ice and 10% sodium carbonate solution. The solid that formed was washed with water, dissolved in methylene chloride, washed with sodium bicarbonate solution, dried, and concentrated. Crystallization of the residue from methanol afforded 3.24 g (93%) of 4-acetoxy-1-ethyl-2-methyl-3-indolecarboxaldehyde (V) as white needles: mp 165–168°; λ_{max} 5.7 (acetoxy), 6.1 (formyl) μ; 252 mμ (ϵ 23,000), 270 (15,000), 343 (8700); nmr δ 10.3 (CHO), 7.30 (three protons, aromatic), 4.18 (two-proton quartet, NCH_2CH_3), 2.72 (three protons, C-2 methyl), 2.48 (three protons, $COCH_3$), 1.35 (three-proton triplet, CH_2CH_3). An analytical sample, recrystallized from methanol, had mp 166–173°.

Anal. Calcd for $C_{14}H_{15}NO_3$ (245.27): C, 68.55; H, 6.16; N, 5.71. Found: C, 68.33; H, 6.40; N, 5.81.

1-Ethyl-4-hydroxy-2-methyl-3-indolecarboxaldehyde (VI). A mixture of 3.14 g (12 mmoles) of 4-acetoxy-1-ethyl-2-methyl-3-indolecarboxaldehyde (V), 200 ml of methanol, and 60 ml of 5% sodium hydroxide solution was stirred and gently warmed until all of the solid dissolved. The resulting solution was cooled, diluted with 200 ml of water, and carefully neutralized with acetic acid. The precipitate that formed was washed with water, dissolved in methylene chloride, shaken with sodium bicarbonate solution, dried, and concentrated. Crystallization of the residue from methanol (charcoal) gave 1.16 g (45%) of 1-ethyl-4-hydroxy-2-methyl-3-indolecarboxaldehyde (VI) as white needles: mp 169–170°; λ_{max} 2.9, 6.25 (hydrogen-bonded formyl) μ; 253 mμ (ϵ 25,000), 270 (15,500), 345 (8800).

Anal. Calcd for $C_{12}H_{13}NO_2$ (203.23): C, 70.91; H, 6.45; N, 6.89. Found: C, 70.88; H, 6.57; N, 7.03.

1-Ethyl-2-methyl-4,7-indoloquinone (IV). To a solution of 1.34 g (5 mmoles) of potassium nitrosodisulfonate in 80 ml of *M*/18 potassium dihydrogen phosphate was added a solution of 218 mg (1.25 mmoles) of 1-ethyl-4-hydroxy-2-methylindole (II).[3] After 1 hr the amber solution was diluted with 500 ml of water and extracted with 200 ml of methylene chloride. This extract was washed with water, dried, and concentrated, and the residue was purified by adsorption chromatography on Florisil[28] with methylene chloride as eluent. The orange eluate afforded on concentration 160 mg (68%) of 1-ethyl-2-methyl-4,7-indoloquinone (IV) as scarlet prisms: mp 86–87°; λ_{max} 6.12 μ; 230 mμ (ϵ 15,300), 254 (11,800), 345 (2500), 440 (2100); nmr δ 6.55 (two protons, C-5 and C-6), 6.39 (C-3 proton), 3.78 (two-proton quartet, NCH_2CH_3), 2.38 (three protons, C-2 methyl), 1.40 (three-proton doublet, NCH_2CH_3). An analytical sample, recrystallized from hexane, had mp 86–87°.

Anal. Calcd for $C_{11}H_{11}NO_2$ (189.21): C, 69.82; H, 5.86; N, 7.40. Found: C, 69.52; H, 5.90; N, 7.44.

After elution of the orange band from the Florisil column just described, the solvent was changed to acetone and a purple band was eluted. Concentration of the eluate and recrystallization of

tories pointed out to us that involvement of the 3-carboxaldehyde group with intermediates in this oxidation might have been responsible for this failure and suggested that a compound (such as XXX) having a 3 substituent at the hydroxymethyl oxidation level might achieve our ends.

(27) A complete survey of the testing results will be published elsewhere.

(28) Florisil is the registered trademark of the Floridin Co. for a magnesia–silica gel adsorbent.

the residue thus obtained from methylene chloride–hexane afforded 28 mg (12%) of purple prisms: mp 103–107°; λ_{max} 6.04 μ; 240 mμ (ϵ 28,000), 355 (4060), 570 (1565); nmr δ 7.17 (doublet, $J_{6,7}$ = 11 cps, C-6 proton), 6.33 (C-3 proton), 5.91 (doublet, $J_{6,7}$ = 11 cps, C-7 proton), 3.95 (two-proton quartet, NCH_2CH_3), 2.25 (three protons, C-2 methyl), 1.37 (three-proton triplet, NCH_2CH_3). Satisfactory analysis could not be obtained on this sample.

4,7-Diacetoxy-1-ethyl-2-methyl-3-indolecarboxaldehyde (VIII). A mixture of 1.13 g (6.0 mmoles) of 1-ethyl-2-methyl-4,7-indoloquinone (IV), 1.1 g (excess) of zinc dust, and 10 ml of acetic anhydride was cooled in an ice bath and treated with 0.5 ml of pyridine.[8] After 30 min at room temperature the resulting mixture was filtered into sodium bicarbonate solution. When the excess anhydride appeared to be hydrolyzed, the mixture was extracted with methylene chloride and this extract was washed with sodium bicarbonate solution, dried, and concentrated. The residual oil was purified by adsorption chromatography on Florisil with ether as solvent. Concentration of the eluate afforded 1.23 g (75%) of 4,7-diacetoxy-1-ethyl-2-methylindole (VII) as yellow oil: λ_{max} 5.68 μ. Without further purification VII was converted to the corresponding 3-formyl derivative.

To an ice-cooled mixture of 690 mg (4.5 mmoles) of phosphorus oxychloride and 5 ml of dimethylformamide was added dropwise a solution of 1.23 g (4.5 mmoles) of VII in 10 ml of dimethylformamide. The mixture was stirred for 60 min at ice-bath temperature and 30 min at room temperature, poured onto ice and 10% sodium carbonate solution, and filtered. The green solid product was washed with water, dissolved in methylene chloride, washed with sodium bicarbonate solution, dried, and concentrated. Scratching induced crystallization of the green oily residue. Recrystallization from methanol afforded 634 mg (85%) of 4,7-diacetoxy-1-ethyl-2-methyl-3-indolecarboxaldehyde (VIII) as bluish white prisms: mp 124–126°; λ_{max} 5.72 (acetate), 6.08 (formyl) μ; 253 mμ (ϵ 22,000), 272 (15,000), 343 (8700).

Anal. Calcd for $C_{16}H_{17}NO_5$ (303.30): C, 63.36; H, 5.65; N, 4.62. Found: C, 63.37; H, 5.59; N, 5.11, 4.58.

In a 15-g scale run a conversion of quinone IV to diacetoxyformylindole VIII in 74% over-all yield was obtained.

1-Ethyl-2-methyl-4,7-indoloquinone-3-carboxaldehyde (IX). A. From VI. To a stirred solution of 1.98 g (7.4 mmoles) of potassium nitrosodisulfonate in 180 ml of *M*/18 potassium dihydrogen phosphate was added a hot solution of 375 mg (1.85 mmoles) of 1-ethyl-4-hydroxy-2-methyl-3-indolecarboxaldehyde (VI) in 50 ml of acetone. The mixture was stirred at 40° for 10 min and treated with an additional 990 mg (3.7 mmoles) of potassium nitrosodisulfonate in 60 ml of *M*/18 potassium dihydrogen phosphate and with 40 ml of hot acetone. After 30 min the mixture was diluted with 400 ml of water and filtered, and the filtrate was extracted with methylene chloride. This extract was washed with water, dried, and concentrated. The residue was purified by adsorption chromatography on Florisil with acetone as solvent. Concentration of the orange eluate gave orange solid which was recrystallized from acetone–hexane to afford 186 mg (46%) of 1-ethyl-2-methyl-4,7-indoloquinone-3-carboxaldehyde (IX) as orange prisms: mp 148–155°; λ_{max} 6.0, 6.1 μ; 246 mμ (ϵ 19,000), 256 (18,500), 265 (15,000), 335 (3000), 430 (3000).

A sample of IX was purified for analysis by partition chromatography on diatomaceous earth with the system hexane–ethyl acetate–methanol–water (50:50:15:6). Concentration of the eluate from the only colored band (orange) gave orange needles, mp 148–158°.

Anal. Calcd for $C_{12}H_{11}NO_3$ (217.22): C, 66.35; H, 5.10; N, 6.45. Found: C, 66.24; H, 5.32; N, 6.56.

B. From VIII. A mixture of 606 mg (2 mmoles) of 4,7-diacetoxy-1-ethyl-2-methyl-3-indolecarboxaldehyde (VIII), 80 mg (2 mmoles) of sodium hydroxide, and 10 ml of methanol was stirred under nitrogen for 10 min and treated with a solution of 1.08 g (4 mmoles) of ferric chloride hexahydrate in 10 ml of 0.2 *N* hydrochloric acid. A methylene chloride extract of the diluted mixture was washed with sodium bicarbonate solution, dried, and concentrated to afford 310 mg (71%) of 1-ethyl-2-methyl-4,7-indoloquinone-3-carboxaldehyde (IX), mp 148–159°, undepressed on admixture of IX prepared from VI, identical in ultraviolet and infrared spectrum with IX prepared from VI: nmr δ 10.50 (CHO), 6.61 (two protons, C-5 and C-6 protons), 4.43 (two-proton quartet, NCH_2CH_3), 2.65 (three protons, C-2 methyl), 1.39 (three-proton triplet, NCH_2CH_3).

1-Ethyl-3-hydroxymethyl-2-methyl-4,7-indoloquinone *n*-Propylcarbamate (Xb). A solution of 675 mg (3.1 mmoles) of 1-ethyl-2-methyl-4,7-indoloquinone-3-carboxaldehyde (IX) in 110 ml of methanol under nitrogen was treated with a solution of 650 mg (excess) of sodium borohydride in 10 ml of methanol. The mixture was warmed to reflux temperature, then stirred at room temperature for 1 hr. Acetone (15 ml) was added, followed after 5 min by a solution of 2.1 g (7.8 mmoles) of ferric chloride hexahydrate in 15 ml of 0.1 *N* hydrochloric acid. The resulting mixture was treated with methylene chloride and water and the organic layer was washed two times with water, dried, and concentrated. A solution of the red semisolid residue in 10 ml of the upper phase and 10 ml of the lower phase of the system heptane–ethyl acetate–methanol–water (50:50:15:6) was mixed with 20 g of Celite[29] and packed atop a column of 250 g of Celite and 125 ml of the lower phase. Elution with the upper phase produced two orange bands on the column. Concentration of the eluate from the second band afforded 125 mg of **1-ethyl-3-hydroxymethyl-2-methyl-4,7-indoloquinone** (Xa) as red solid: mp 75–100°; λ_{max} 2.85 (OH), 6.1 (quinone carbonyls) μ; 232 mμ (ϵ 20,000), 257 (13,500), 350 (3300), 445 (3300); nmr δ 6.61 (two protons, C-5 and C-6 protons), 4.65 (two-proton doublet, J = 6 cps, changes to singlet on addition of D_2O, CH_2OH), 4.37 (two-proton quartet, NCH_2CH_3), 4.25 (doublet, J = 6 cps, disappears on addition of D_2O, OH), 2.29 (three protons, C-2 methyl), 1.33 (three-proton triplet, NCH_2CH_3). Without further purification Xa was converted to the corresponding *n*-propyl carbamate.

A mixture of 105 mg of 1-ethyl-3-hydroxymethyl-2-methyl-4,7-indoloquinone (Xa) and 3 ml of *n*-propyl isocyanate was heated at reflux temperature for 3 hr. The excess isocyanate was removed under reduced pressure and the residual orange oil was treated with ether and hexane. This procedure afforded 46 mg (31%) of 1-ethyl-3-hydroxymethyl-2-methyl-4,7-indoloquinone *n*-propylcarbamate (Xb) as orange crystals: mp 93–96°; λ_{max} 2.98, 5.85 (carbamate), 6.12 (quinone) μ; 232 mμ (ϵ 14,200), 255 (9650), 340 (2610), 437 (1010); nmr δ 6.49 (two protons, C-5 and C-6 protons), 5.25 (two protons, CH_2O), 4.35 (two-proton quartet, NCH_2CH_3), 3.15 (two-proton quartet, $OCNHCH_2CH_2$), 2.35 (three protons, C-2 methyl), 1.45 (two-proton pentuplet, $CH_2CH_2CH_3$), 1.35 (three-proton triplet, NCH_2CH_3), 0.90 (three-proton triplet, $CH_2CH_2CH_3$). The NH proton could not be distinguished from the base line. In an attempt to prepare an analytical sample from Xb by recrystallization from methanol partial decomposition apparently occurred and the product was contaminated with what is probably the corresponding diindolylmethane[10,11] ($C_{23}H_{22}N_2O_4$). Decreased carbamate absorption in the infrared was noted. The resulting orange crystalline solid had indefinite decomposition at 172–200°.

Anal. Calcd for $C_{16}H_{20}N_2O_4$ (304.34): C, 63.14; H, 6.62; N, 9.21. Found: C, 65.98; H, 6.63; N, 8.34. Calcd for 60% $C_{16}H_{20}N_2O_4$ and 40% $C_{23}H_{22}N_2O_4$: C, 66.10; H, 6.24; N, 8.40.

Treatment of 8.0 mg of 1-ethyl-3-hydroxymethyl-2-methyl-4,7-indoloquinone (Xa) with excess sodium acetate and acetic anhydride at steam-bath temperature for 1.5 hr afforded 7.0 mg (73%) of **3-acetoxymethyl-1-ethyl-2-methyl-4,7-indoloquinone** (Xc) as orange crystals: mp 103–105°; λ_{max} 5.70 (acetate), 6.12 (quinone) μ; 230 mμ (ϵ 15,400), 255 (11,800), 345 (2610), 437 (1020).

5,6-Dibromo-1-ethyl-2-methyl-4,7-indoloquinone-3-carboxaldehyde (XI). A solution of 1.085 g (5 mmoles) of 1-ethyl-2-methyl-4,7-indoloquinone-3-carboxaldehyde (IX) and 3.28 g of sodium acetate in 30 ml of acetic acid was cooled in a water bath and treated with 1.60 g (10 mmoles) of bromine. After the mixture was stirred for 1 day at room temperature, the red solid that separated was collected, washed with water, and dissolved in methylene chloride. This solution was washed with sodium bicarbonate solution, dried, and concentrated, and the residue was recrystallized from methylene chloride–hexane to afford 470 mg (25%) of 5,6-dibromo-1-ethyl-2-methyl-4,7-indoloquinone-3-carboxaldehyde (XI) as red needles: mp 200–210°; λ_{max} 6.08 μ; 231 mμ (ϵ 18,000), 301 (15,200), 357 (4000), 475 (2800).

Anal. Calcd for $C_{12}H_9Br_2NO_3$ (375.03): C, 38.43; H, 2.44; Br, 42.61; N, 3.73. Found: C, 38.43; H, 2.71; Br, 42.91; N, 3.65.

Dilution with water of the filtrate from the above reaction mixture afforded a small quantity of monobrominated product (probably a mixture of isomeric 5- and 6-bromo-1-ethyl-2-methyl-4,7-indoloquinone-3-carboxaldehydes) as orange solid, mp 114–120°.

Anal. Calcd for $C_{12}H_{10}BrNO_3$ (296.12): C, 48.67; H, 3.40; Br, 26.99. Found: C, 48.11; H, 3.22; Br, 26.40.

When a cooled solution of 4.34 g (20 mmoles) of IX and 13.12 g of sodium acetate in 120 ml of acetic acid was treated initially with

(29) Celite is the registered trademark of the Johns-Manville Corp. for diatomaceous earth products.

6.40 g (40 mmoles) of bromine, and with additional 3.20-g (20 mmoles) portions of bromine after 6 hr and 2 days, work-up of the mixture after 4 days by the above procedure gave 6.24 g (83%) of dibromoquinone XI, mp 200–210°.

5-Bromo-1-ethyl-6-hydroxy-2-methyl-4,7-indoloquinone-3-carboxaldehyde (XII) and 6-Bromo-1-ethyl-5-hydroxy-2-methyl-4,7-indoloquinone-3-carboxaldehyde (XIII). A suspension of 303 mg (0.81 mmole) of 5,6-dibromo-1-ethyl-2-methyl-4,7-indoloquinone-3-carboxaldehyde (XI) in 50 ml of methanol was treated with a solution of 129.6 mg (3.24 mmoles) of sodium hydroxide in 10 ml of water. The mixture was warmed briefly on a steam bath, kept at room temperature for 2.5 hr, diluted with 200 ml of water, and extracted with methylene chloride. The purple aqueous phase was acidified with dilute hydrochloric acid and the resulting orange solution was extracted with methylene chloride. This extract was washed with water, dried, and concentrated. The orange solid residue (102 mg, 41%) was recrystallized from benzene, affording 90 mg of a mixture of 5-bromo-1-ethyl-6-hydroxy-2-methyl-4,7-indoloquinone-3-carboxaldehyde (XII) and 6-bromo-1-ethyl-5-hydroxy-2-methyl-4,7-indoloquinone-3-carboxaldehyde (XIII): mp 205–212° dec; λ_{max} 3.0 (broad), 6.07 μ. Although satisfactory combustion analyses could not be obtained for this mixture of isomers, it was converted to the corresponding methoxyquinone isomers, each of which, after resolution, gave satisfactory analytical data.

5-Bromo-1-ethyl-6-methoxy-2-methyl-4,7-indoloquinone-3-carboxaldehyde (XV) and 6-Bromo-1-ethyl-5-methoxy-2-methyl-4,7-indoloquinone-3-carboxaldehyde (XIV). To a solution of 653 mg (2.1 mmoles) of a mixture of 5-bromo-1-ethyl-6-hydroxy-2-methyl-4,7-indoloquinone-3-carboxaldehyde (XII) and 6-bromo-1-ethyl-5-hydroxy-2-methyl-4,7-indoloquinone-3-carboxaldehyde (XIII) in 120 ml of methylene chloride was added a solution of diazomethane (prepared from 472 mg (3.2 mmoles) of N-nitroso-N-methyl-N′-nitroguanidine) in ether. After 30 min the solution was extracted with 2% sodium bicarbonate solution, dried, and concentrated. The residue was dissolved in 25 ml of the upper phase and 25 ml of the lower phase of the system heptane–methyl cellosolve, mixed with 50 g of Celite, and packed atop a column prepared from 300 ml of the lower phase and 600 g of Celite. Elution with the upper phase gave in hold-back volumes 5.6–7.2 (960 ml per hold-back volume), after concentration and crystallization of the residue from methanol, 155 mg of 6-bromo-1-ethyl-5-methoxy-2-methyl-4,7-indoloquinone-3-carboxaldehyde (XIV) as orange plates: mp 131–135°; λ_{max} 5.93, 6.05 μ; 218 mμ (ϵ 20,000), 245 (shoulder, 11,700), 265 (9500), 304 (10,800), 340 (5900), 450 (650).

Anal. Calcd for $C_{13}H_{12}BrNO_4$ (326.16): C, 47.86; H, 3.71; Br, 24.50. Found: C, 47.56; H, 3.84; Br, 24.23.

In one instance, attempted recrystallization of XIV from methanol afforded the corresponding dimethyl acetal, red needles: mp 100–101°; λ_{max} 3.37 (strong), 6.0, 6.08 μ; 233 mμ (ϵ 16,000), 298 (13,000), 350 (2700), 465 (1420).

Anal. Calcd for $C_{15}H_{18}BrNO_5$ (372.22): C, 48.43; H, 4.87; N, 3.77; Br, 21.72. Found: C, 48.33; H, 5.12; N, 3.82; Br, 21.72.

Treatment of this dimethyl acetal with aqueous methanol containing hydrochloric acid regenerated the parent aldehyde XV, identical in melting point, mixture melting point, and infrared spectrum with the above-described sample.

Concentration of the eluate in hold-back volumes 7.4–8.4 gave, after recrystallization from methanol, 172 mg of 5-bromo-1-ethyl-6-methoxy-2-methyl-4,7-indoloquinone-3-carboxaldehyde (XV) as orange-red needles: mp 180–181°; λ_{max} 5.93, 6.05 μ; 218 mμ (ϵ 20,000), 244 (11,600), 268 (9600), 302 (9600), 340 (5900), 450 (800).

Anal. Calcd for $C_{13}H_{12}BrNO_4$ (326.16): C, 47.84; H, 3.71; Br, 24.50. Found: C, 48.21; H, 4.07; Br, 24.12.

6-Bromo-1-ethyl-3-hydroxymethyl-5-methoxy-2-methyl-4,7-indoloquinone *n*-Propylcarbamate (XVIIIb) and 1-Ethyl-3-hydroxymethyl-5-methoxy-2-methyl-4,7-indoloquinone *n*-Propylcarbamate (XVIb). A. Directly from XIV. A suspension of 343 mg of 6-bromo-1-ethyl-5-methoxy-2-methyl-4,7-indoloquinone-3-carboxaldehyde (XIV) in 100 ml of methanol was stirred under nitrogen for 10 min and treated with 340 mg (excess) sodium borohydride in 10 ml of ethanol. The mixture was warmed briefly on a water bath and then stirred at room temperature for 30 min. Acetone (5 ml) was added, followed after 5 min by a solution of 540 mg of ferric chloride hexahydrate in 20 ml of 0.1 *N* hydrochloric acid. The resulting mixture was treated with water and methylene chloride and the organic layer was washed with sodium bicarbonate solution, dried, and concentrated. The orange semisolid residue (292 mg) was dissolved in 6.5 ml of the upper phase and 6.5 ml of the lower phase of the system methanol–hexane, mixed with 13 g of Celite, and packed atop a column prepared from 200 g of Celite and 100 ml of the lower phase. Elution with the upper phase produced three colored bands, which were collected separately. Concentration of the eluate from the second band gave 80 mg (23%) of **6-bromo-1-ethyl-3-hydroxymethyl-5-methoxy-2-methyl-4,7-indoloquinone** (XVIIIa) as red solid, mp 114–138°.

Without further purification XVIIIa was converted to the *n*-propylcarbamate. A mixture of 80 mg of XVIIIa and 3 ml of *n*-propyl isocyanate was heated on a steam bath for 3 hr and concentrated; the residue was crystallized from ether. This procedure afforded 55 mg (55%) of 6-bromo-1-ethyl-3-hydroxymethyl-5-methoxy-2-methyl-4,7-indoloquinone *n*-propylcarbamate (XVIIIb) as orange needles: mp 144–146°; λ_{max} 3.0, 5.92 (carbamate), 6.04 (quinone) μ; 233 mμ (ϵ 16,100), 300 (13,000), 360 (3500), 475 (870).

Anal. Calcd for $C_{17}H_{21}BrN_2O_5$ (413.28): C, 49.69; H, 5.12; Br, 19.33. Found: C, 50.24; H, 5.85; Br, 19.45.

Concentration of the eluate from the third band gave 70 mg (24%) of **1-ethyl-3-hydroxymethyl-5-methoxy-2-methyl-4,7-indoloquinone** (XVIa) as red needles, mp 164–165°. This product was converted directly to the *n*-propylcarbamate. Thus, a mixture of 70 mg of XVIa and 5 ml of *n*-propyl isocyanate was heated on a steam bath for 3 hr and concentrated, and the residue was crystallized from ether–hexane. This procedure afforded 36 mg (41%) of 1-ethyl-3-hydroxymethyl-5-methoxy-2-methyl-4,7-indoloquinone *n*-propylcarbamate (XVIb) as yellow needles: mp 144–146°; λ_{max} 2.9, 5.95, 6.08 μ; 229 mμ (ϵ 15,000), 299 (15,200), 343 (2500), 450 (1170).

Anal. Calcd for $C_{17}H_{22}N_2O_5$ (334.26): C, 61.06; H, 6.63; N, 8.38. Found: C, 61.29; H, 6.94; N, 8.39.

Concentration of the eluate from the first band gave a red solid that showed a positive Beilstein test for halogen, but had no absorption in the OH stretching region of the infrared spectrum. It failed to give a carbamate on treatment with *n*-propyl isocyanate. This solid was not further investigated.

B. From the Hydroquinone of XIV. A solution of 116 mg (0.36 mmole) of 6-bromo-1-ethyl-5-methoxy-2-methyl-4,7-indoloquinone-3-carboxaldehyde (XIV) in methylene chloride was shaken with excess sodium dithionite in water until the organic layer was nearly decolorized. The organic layer was dried and concentrated. A suspension of the residual hydroquinone (XXII) in methanol was treated with 120 mg (excess) of sodium borohydride, under nitrogen, and the mixture was warmed briefly on a water bath, stirred for 30 min at room temperature, treated with excess acetone and 200 mg of ferric chloride hexahydrate in 10 ml of 0.1 *N* hydrochloric acid, and worked up as described in part A. Partition chromatography on 80 g of Celite as described above afforded 22 mg of **6-bromo-1-ethyl-3-hydroxymethyl-5-methoxy-2-methyl-4,7-indoloquinone** (XVIIIa), identical in infrared absorption spectrum with the sample obtained in part A, and 37 mg (42%) of **1-ethyl-3-hydroxymethyl-5-methoxy-2-methyl-4,7-indoloquinone** (XVIa), identical in infrared absorption spectrum, melting point, and mixture melting point with the sample obtained in part A. A third solid product was also obtained.

Treatment of hydroquinone XXII (prepared by sodium dithionite reduction of XIV as described above) in methanol with ferric chloride in 0.1 *N* hydrochloric acid afforded an almost quantitative return of XIV, identical in infrared spectrum, melting point, and mixture melting point with the authentic sample.

5-Bromo-1-ethyl-3-hydroxymethyl-6-methoxy-2-methyl-4,7-indoloquinone *n*-Propylcarbamate (XIXb) and 1-Ethyl-3-hydroxymethyl-6-methoxy-2-methyl-4,7-indoloquinone *n*-Propylcarbamate (XVIIb). A suspension of 652 mg (2 mmoles) of 5-bromo-1-ethyl-6-methoxy-2-methyl-4,7-indoloquinone-3-carboxaldehyde (XV) in 250 ml of methanol was stirred under nitrogen for 15 min, warmed on a water bath, and treated with 650 mg (excess) of sodium borohydride in 40 ml of ethanol. The mixture was stirred at room temperature for 1 hr and treated with 10 ml of acetone, followed after 5 min by a solution of 1.24 g (4 mmoles) of ferric chloride hexahydrate in 20 ml of 0.1 *N* hydrochloric acid. The mixture was treated with water and methylene chloride and the organic layer was washed with sodium bicarbonate solution, dried, and concentrated. A solution of the red oily residue in 5 ml of the upper phase and 5 ml of the lower phase of the system methanol–hexane was mixed with 10 g of Celite, packed atop a column prepared from 200 g of Celite and 100 ml of the lower phase, and eluted with the

upper phase. Four colored bands developed and were separately collected and concentrated. The third band (dark red) afforded 192 mg (29%) of **5-bromo-1-ethyl-3-hydroxymethyl-6-methoxy-2-methyl-4,7-indoloquinone** (XIXa) as red needles, mp 137–138.5°. This compound was converted directly to the *n*-propylcarbamate. Thus, a mixture of 80 mg of XIXa and 3.5 ml of *n*-propyl isocyanate was heated on a steam bath for 3 hr and concentrated, and the residue was crystallized from ether. This procedure afforded 69 mg (68%) of 5-bromo-1-ethyl-3-hydroxymethyl-6-methoxy-2-methyl-4,7-indoloquinone *n*-propylcarbamate (XIXb) as orange plates: mp 162.5–164°; λ_{max} 2.95, 5.92 (carbamate), 6.04 (quinone) μ; 229 mμ (ϵ 16,200), 298 (13,000), 350 (2720), 465 (1450).

Anal. Calcd for $C_{17}H_{21}BrN_2O_5$ (413.28): C, 49.69; H, 5.12; Br, 19.33. Found: C, 49.23; H, 5.02; Br, 19.53.

The fourth band (orange) gave 74 mg (15%) of **1-ethyl-3-hydroxymethyl-6-methoxy-2-methyl-4,7-indoloquinone** (XVIIa) as orange solid, λ_{max} 2.83, 6.10 μ. This solid was converted directly to the *n*-propylcarbamate. Thus, a mixture of 74 mg of XVIIa and 5 ml of *n*-propyl isocyanate was heated on a steam bath for 3 hr and concentrated; the residue was crystallized from ether and recrystallized from methylene chloride–hexane. This procedure gave 41 mg (41%) of 1-ethyl-3-hydroxymethyl-6-methoxy-2-methyl-4,7-indoloquinone *n*-propylcarbamate (XVIIb) as yellow granules: mp 136–138°; λ_{max} 2.9, 5.9 (carbamate), 6.10 (quinone) μ; 228 mμ (ϵ 14,900), 287 (12,700), 340 (4340), 445 (1340), identical in infrared absorption spectrum and melting point with, and not depressing the melting point on admixture with samples prepared from XXV and XXX (see below).

Anal. Calcd for $C_{17}H_{22}N_2O_5$ (334.36): C, 61.06; H, 6.63; N, 8.34. Found: C, 61.22; H, 6.89; N, 8.34.

The first band (red) gave 160 mg of a red solid that showed a positive Beilstein test. It had no OH stretching absorption in the infrared spectrum and failed to give an *n*-propylcarbamate. The second band gave a trace of tan solid which was not further investigated.

Other Preparations of 1-Ethyl-3-hydroxymethyl-6-methoxy-2-methyl-4,7-indoloquinone *n*-Propylcarbamate (XVIIb). A. From the Hooker Oxidation of 2,6-Dimethyl-1-ethyl-5-hydroxy-3-hydroxymethyl-4,7-indoloquinone Methylcarbamate. A solution of 860 mg (28 mmoles) of 2,6-dimethyl-1-ethyl-5-hydroxy-3-hydroxymethyl-4,7-indoloquinone methylcarbamate (XXX) in 43 ml of 0.1 *N* sodium hydroxide was treated at 5° with a solution of 600 mg (38 mmoles) of potassium permanganate in 36 ml of 1.0 *N* sodium hydroxide. (Compound XXX was prepared by stirring a suspension of 2,6-dimethyl-1-ethyl-3-hydroxymethyl-5-methoxy-4,7-indoloquinone methylcarbamate[2a] in 0.1 *N* hydrochloric acid for 8 hr, mp 190–192°; *Anal.* Found: C, 58.76; H, 6.11; N, 9.16; this experiment was performed by Mr. J. F.Poletto of these laboratories.) After 25 min manganese dioxide was removed by filtration and the filtrate was kept at room temperature for 4 hr, carefully acidified with dilute hydrochloric acid, and extracted with methylene chloride. This extract was washed two times with brine, dried, and concentrated. The orange semisolid residue (240 mg) was directly methylated with diazomethane.

An ice-cooled solution of 120 mg of the orange semisolid described in the preceding sentence in 5 ml of methylene chloride was treated with ethereal diazomethane (prepared in the usual manner from 110 mg of N-nitroso-N-methyl-N′-nitroguanidine). After 1 hr the solution was evaporated and the residual orange solid was dissolved in 7.5 ml of the upper phase and 7.5 ml of the lower phase of the system methanol–heptane, mixed with 15 g of Celite, and packed atop a column prepared from 150 g of Celite and 75 ml of the lower phase. Elution with the upper phase produced a single, deep yellow band. Concentration of the eluate from this band gave an orange solid which afforded, on recrystallization from methylene chloride–hexane, 37 mg (11% from XXX) of **1-ethyl-3-hydroxymethyl-6-methoxy-2-methyl-4,7-indoloquinone** (XVIIa) as orange solid, mp 165–166.5°, identical in infrared spectrum with XVIIa prepared from XV and from XXV. A mixture of 24 mg of this solid and 1.0 ml of *n*-propyl isocyanate was heated on a steam bath for 3 hr. The excess isocyanate was removed under reduced pressure and the oily residue was treated with ether. This procedure gave 30 mg of 1-ethyl-3-hydroxymethyl-6-methoxy-2-methyl-4,7-indoloquinone *n*-propylcarbamate (XVIIb) as yellow needles, mp 136–138°; melting point undepressed on admixture of XVIIb prepared from XV or from XXV; infrared spectrum identical with XVIIb prepared from XV or from XXV.

B. From 1-Ethyl-6-methoxy-2-methyl-4,7-indoloquinone-3-carboxaldehyde (XXVIII). A suspension of 110 mg (0.45 mmole) of 1-ethyl-6-methoxy-2-methyl-4,7-indoloquinone-3-carboxaldehyde (XXVIII) in 20 ml of methanol, under nitrogen, was treated with a slurry of 125 mg (excess) of sodium borohydride in 5 ml of ethanol. The mixture was heated at reflux temperature for 10 min, stirred at room temperature for 35 min, and treated with 2.5 ml of acetone. A solution of 270 mg (1 mmole) of ferric chloride hexahydrate in 15 ml of 0.1 *N* hydrochloric acid was added and the mixture was treated with methylene chloride and water. The organic layer was washed with 2% sodium bicarbonate solution, dried, and concentrated. The orange solid residue (36 mg) had an infrared absorption spectrum identical with that of XVIIa prepared from XV and from XXX. Without further purification it was converted to the corresponding *n*-propylcarbamate. Thus, a mixture of 36 mg of this orange solid residue and 0.5 ml of *n*-propyl isocyanate was heated on a steam bath for 3 hr. The excess isocyanate was removed under reduced pressure and the residue was crystallized from ether. This procedure afforded 23 mg of 1-ethyl-3-hydroxymethyl-6-methoxy-2-methyl-4,7-indoloquinone *n*-propylcarbamate (XVIIb) as yellow solid, mp 136–138°; undepressed on admixture of XVIIb prepared from XXX; infrared absorption spectrum identical with that of XVIIb prepared from XXX.

5,6-Dibromo-1-ethyl-3-hydroxymethyl-2-methyl-4,7-indoloquinone *n*-Propylcarbamate (XXIVb). A suspension of 442 mg (1.18 mmoles) of 5,6-dibromo-1-ethyl-2-methyl-4,7-indoloquinone-3-carboxaldehyde (XI) in 75 ml of methanol under nitrogen was treated with a solution of 350 mg (excess) sodium borohydride in 20 ml of methanol. The mixture was heated briefly to boiling, stirred at room temperature for 30 min, and treated with 10 ml of acetone. After 5 min a solution of 640 mg (2.36 mmoles) of ferric chloride hexahydrate in 9 ml of 0.1 *N* hydrochloric acid was added and the mixture was treated with water and methylene chloride. The layer was washed three times with water, dried, and concentrated, and the red solid residue was purified by partition chromatography using the same column and solvent system described for XIa. Only one colored band (red) formed on the column and concentration of the eluate from this band gave 63 mg of impure 5,6-dibromo-1-ethyl-3-hydroxymethyl-2-methyl-4,7-indoloquinone (XXIVa) as dark red prisms, mp 115–122°. A mixture of these prisms and 2 ml of *n*-propyl isocyanate was heated at steam-bath temperature for 3 hr, the excess isocyanate was removed under reduced pressure, and the semisolid residue was triturated with ether and hexane. This procedure afforded 41 mg of 5,6-dibromo-1-ethyl-3-hydroxymethyl-2-methyl-4,7-indoloquinone *n*-propylcarbamate (XXIVb), contaminated with the corresponding monobromoquinone carbamate, as orange plates: mp 187–191°; λ_{max} 2.9, 5.95 (carbamate), 6.05 (quinone) μ; 229 mμ (ϵ 16,800), 292 (13,200), 360 (3400), 468 (2270).

Anal. Calcd for $C_{16}H_{18}Br_2N_2O_4$ (462.15): C, 41.59; H, 3.92; Br, 34.57. Found: C, 44.93; H, 4.59; Br, 30.56. Calcd for a mixture containing 67% of XXIVb and 33% of monobromoquinone *n*-propylcarbamate ($C_{16}H_{19}BrN_2O_4$): C, 44.45; H, 4.58; Br, 30.00.

5-Amino-6-bromo-1-ethyl-3-hydroxymethyl-2-methyl-4,7-indoloquinone *n*-Propylcarbamate (XX). A solution of 40 mg of 6-bromo-1-ethyl-3-hydroxymethyl-5-methoxy-2-methyl-4,7-indoloquinone *n*-propylcarbamate (XVIIIb) in 25 ml of methanol was treated with excess anhydrous ammonia. The mixture was kept at room temperature overnight and concentrated, and the residue was taken up in methylene chloride, washed with water, dried, and concentrated. Crystallization of the residue from methylene chloride–hexane gave 30 mg (78%) of 5-amino-6-bromo-1-ethyl-3-hydroxymethyl-2-methyl-4,7-indoloquinone *n*-propylcarbamate (XX) as purple granules: mp 136–142°; λ_{max} 2.9, 5.88 (carbamate), 6.24 (quinone) μ; 241 mμ (ϵ 20,400), 316 (11,300), 358 (7500), 520 (1800).

Anal. Calcd for $C_{16}H_{20}BrN_3O_4$ (398.27): C, 48.25; H, 5.06; Br, 20.06. Found: C, 49.00; H, 5.13; Br, 19.86.

6-Amino-5-bromo-1-ethyl-3-hydroxymethyl-2-methyl-4,7-indoloquinone *n*-Propylcarbamate (XXIa). A solution of 40 mg of 5-bromo-1-ethyl-3-hydroxymethyl-6-methoxy-2-methyl-4,7-indoloquinone *n*-propylcarbamate (XIXb) in 20 ml of methanol was treated with excess anhydrous ammonia. After 4 hr the mixture was concentrated and the residue was treated with methylene chloride and water. The organic layer was dried and concentrated and the residue was crystallized from ether. This procedure afforded 36 mg (92%) of 6-amino-5-bromo-1-ethyl-3-hydroxymethyl-2-methyl-4,7-indoloquinone (XXIa) as pink needles: mp 178° dec; λ_{max} 2.9, 5.93 (carbamate), 6.00 (quinone) μ; 238 mμ (ϵ20,500), 317 (10,400), 360 (9150), 520 (600).

Anal. Calcd for $C_{16}H_{20}BrN_3O_4$ (398.27): C, 48.25; H, 5.06; Br, 20.06. Found: C, 48.39; H, 5.19; Br, 19.89.

5-Bromo-1-ethyl-3-hydroxymethyl-2-methyl-6-methylamino-4,7-indoloquinone *n*-Propylcarbamate (XXIb). A solution of 50 mg of 5-bromo-1-ethyl-3-hydroxymethyl-6-methoxy-2-methyl-4,7-indoloquinone *n*-propylcarbamate (XIXb) in 10 ml of methylene chloride was treated with excess anhydrous methylamine. After 2 hr the solution was washed with sodium bicarbonate solution and water, dried, and concentrated. Crystallization of the residue from methylene chloride–hexane gave 5-bromo-1-ethyl-3-hydroxymethyl-2-methyl-6-methylamino-4,7-indoloquinone *n*-propylcarbamate (XXIb) as purple plates: mp 168–169° dec; λ_{max} 2.9, 5.97 (carbamate), 6.08 (quinone) μ; 249 mμ (ϵ 17,300), 316 (12,000), 350 (6000), 550 (1530).

Anal. Calcd for $C_{17}H_{22}BrN_3O_4$ (412.29): C, 49.51; H, 5.38; N, 10.19; Br, 19.04. Found: C, 49.27; H, 5.57; N, 9.50; Br, 19.43.

1-Ethyl-2-methyl-5-*p*-toluenethio-4,7-indoloquinone-3-carboxaldehyde (XXVII), 1-Ethyl-2-methyl-6-*p*-toluenethio-4,7-indoloquinone-3-carboxaldehyde (XXV), and 5,6-Bis-*p*-toluenethio-1-ethyl-2-methyl-4,7-indoloquinone-3-carboxaldehyde (XXIX). A. With Excess *p*-Toluenethiol. A suspension of 4.34 g (20 mmoles) of 1-ethyl-2-methyl-4,7-indoloquinone-3-carboxaldehyde (IX) in 200 ml of ethanol was treated with 1.87 g (15 mmoles) of *p*-toluenethiol and stirred at room temperature for 66 hr. A solution of 8.10 g (30 mmoles) of ferric chloride hexahydrate in 20 ml of ethanol was added, followed after 20 min by an additional 1.24 g (10 mmoles) of *p*-toluenethiol. After 16 hr, 4.05 g (15 mmoles) of ferric chloride hexahydrate in 10 ml of ethanol was added, and the mixture was diluted with water and extracted with methylene chloride. This extract was washed with water, dried, and concentrated, and the residue was treated with ether. The orange ether solution was separated from a brown solid residue. Washing of this residue with methanol left as insoluble brown solid 1.47 g (16%) of bis-*p*-toluenethio-1-ethyl-2-methyl-4,7-indoloquinone-3-carboxaldehyde (XXIX): mp 204°; λ_{max} 6.03 μ; nmr δ 10.37 (CHO), 7.27 (four-proton doublet, J = 9 cps, C_6H_3HS), 7.10 (four-proton doublet, J = 9 cps, $CH_3C_6H_3H$), 4.31 (two-proton quartet, NCH_2CH_3), 2.59 (three protons, C-2 methyl), 2.33 (six protons, $CH_3C_6H_4$), 1.25 (three-proton triplet, NCH_2CH_3).

Anal. Calcd for $C_{26}H_{23}NO_3S_2$ (461.58): C, 67.66; H, 5.02; S, 13.90. Found: C, 67.40, 67.81; H, 5.11, 4.91; S, 13.79.

Partial concentration of the orange ether solution gave 485 mg (7.2%) of a red, crystalline solid. Recrystallization of this solid from methanol afforded 245 mg of 1-ethyl-2-methyl-5-*p*-toluenethio-4,7-indoloquinone-3-carboxaldehyde (XXVII) as red needles: mp 175–178°, λ_{max} 6.10 μ.

Anal. Calcd for $C_{19}H_{17}NO_3S$ (339.40): C, 67.23; H, 5.05; S, 9.45. Found: C, 67.50; H, 5.38; S, 9.31.

Complete concentration of the ether solution gave a dark tar that was purified by adsorption chromatography on a column of Florisil (350 times 20 mm) with methylene chloride containing 5% acetone as eluent. Concentration of the eluate gave a brown oil that crystallized on treatment with ether, affording in several crops 2.93 g (43%) of 1-ethyl-2-methyl-6-*p*-toluenethio-4,7-indoloquinone-3-carboxaldehyde (XXV). Recrystallization from methanol gave dark red prisms: mp 189–190°, λ_{max} 6.10 μ.

Anal. Calcd for $C_{19}H_{17}NO_3S$ (339.40): C, 67.23; H, 5.05; N, 4.13; S, 9.45. Found: C, 67.16; H, 5.30; N, 4.03; S, 9.33.

B. With 1 Equiv of *p*-Toluenethiol. A suspension of 434 mg (2 mmoles) of 1-ethyl-2-methyl-4,7-indoloquinone-3-carboxaldehyde (IX) in 20 ml of ethanol was treated with 248 mg (2 mmoles) of *p*-toluenethiol. After 1 hr the mixture was treated with 540 mg (2 mmoles) of ferric chloride hexahydrate in ethanol. The mixture was stirred for 16 hr at room temperature, diluted with water, and extracted with methylene chloride. This extract was washed with water, dried, and concentrated. The red-brown solid residue (548 mg, 82%) had an infrared absorption spectrum that indicated a mixture of *ca.* 75% of 1-ethyl-2-methyl-6-*p*-toluenethio-4,7-indoloquinone-3-carboxaldehyde (XXV) and 25% of 1-ethyl-2-methyl-5-*p*-toluenethio-4,7-indoloquinone-3-carboxaldehyde (XXVII). This ratio of isomers followed from comparison of the intensity of the infrared absorption band at 9.0 μ (present in XXVII, but not in XXV) with the band at 8.1 μ (present in both isomers), the pure isomers obtained in part A serving as reference standards. No bis-*p*-toluenethio derivative (XXIX) was noted in the above-mentioned infrared spectrum or after resolution of the mixture by fractional crystallization.

1-Ethyl-6-methoxy-2-methyl-4,7-indoloquinone-3-carboxaldehyde (XXVIII). A mixture of 990 mg of 1-ethyl-2-methyl-6-*p*-toluenethio-4,7-indoloquinone-3-carboxaldehyde (XXV), 160 ml of methanol, 22 ml of water, and 10 ml of 10% sodium hydroxide solution was heated on a steam bath for 10 min, diluted with 1 l. of water, and extracted with methylene chloride. The aqueous phase was acidified with hydrochloric acid and extracted with methylene chloride. This extract was shaken with 2% sodium bicarbonate solution and the resulting blue aqueous layer was separated, acidified, and extracted with methylene chloride. The last-mentioned extract was dried and concentrated, and the residue was treated with ether. This procedure afforded 700 mg of 1-ethyl-3-formyl-6-hydroxy-2-methyl-4,7-indoloquinone (XXVI) as orange prisms that did not melt below 320°; λ_{max} 3.1 (broad), 6.20 μ. It was not possible to purify this product by crystallization or partition chromatography; therefore, it was converted directly to the corresponding 6-methoxyquinone.

A solution of 354 mg of 1-ethyl-3-formyl-6-hydroxy-2-methyl-4,7-indoloquinone (XXVI) in 15 ml of methylene chloride was treated with ethereal diazomethane (prepared in the usual manner from 325 mg of N-nitroso-N-methyl-N′-nitroguanidine). After 1 hr the mixture was concentrated and the semisolid residue was dissolved in 20 ml of the upper and 20 ml of the lower phases of the system methanol–heptane, mixed with 40 g of Celite, and packed atop a column prepared from 150 g of Celite and 75 ml of the lower phase. Elution with the upper phase gave first a red band and concentration of the eluate from this band gave a small amount of red oil. Concentration of eluate from the second (yellow) band gave orange crystals. Recrystallization from methanol afforded 67 mg (10% from XXV) of 1-ethyl-6-methoxy-2-methyl-4,7-indoloquinone-3-carboxaldehyde (XXVIII) as orange needles: mp 178–182°, λ_{max} 6.05 μ.

Anal. Calcd for $C_{13}H_{13}NO_4$ (247.24): C, 63.15; H, 5.30; N, 5.67. Found: C, 63.08; H, 5.93; N, 6.20.

3-Diacetoxymethyl-1-ethyl-2-methyl-4,7-indoloquinone (iii). A mixture of 217 mg (1 mmole) of 1-ethyl-2-methyl-4,7-indoloquinone-3-carboxaldehyde (IX), 4 ml of acetic anhydride, and 1 drop of concentrated sulfuric acid was stirred 30 hr and poured into sodium bicarbonate solution. After the excess anhydride hydrolyzed the mixture was extracted with methylene chloride and this extract was washed with water, dried, and concentrated. Purification of the residue by adsorption chromatography on Florisil with methylene chloride as eluent gave an orange oil that crystallized on treatment with methanol. Recrystallization from methanol afforded 150 mg (47%) of 3-acetoxymethyl-1-ethyl-2-methyl-4,7-indoloquinone (iii) as orange prisms: mp 137–138°; λ_{max} 5.70 (acetate), 6.05 (quinone) μ; 230 mμ (ϵ 14,100), 255 (9600), 328 (2600), 425 (1000).

Anal. Calcd for $C_{16}H_{17}NO_6$ (319.30): C, 60.18; H, 5.37; N, 4.39. Found: C, 60.45; H, 5.66; N, 4.51.

Hooker Oxidation of 2,6-Dimethyl-1-ethyl-5-hydroxy-4,7-indoloquinone-3-carboxaldehyde. An ice-cooled solution of 494 mg (2 mmoles) of 2,6-dimethyl-1-ethyl-3-formyl-5-hydroxy-4,7-indoloquinone[2a] in 30 ml of 0.1 *N* sodium hydroxide was treated with a solution of 420 mg (2.66 mmoles) of potassium permanganate in 25 ml of 1.0 *N* sodium hydroxide. After 20 min the mixture was filtered. The filtrate was kept at room temperature for 4 hr, acidified, and extracted with methylene chloride. This extract was washed with water, dried, and concentrated and the residual solid was washed with ether. In this manner was obtained 173 mg of red-orange prisms that did not melt below 320°: λ_{max} 2.9, 6.10 μ.

Anal. Calcd for $C_{12}H_{11}NO_4$ (233.22): C, 61.80; H, 4.75; N, 6.01. Found: C, 61.60; H, 4.99; N, 6.10; mol wt, 817.

5-Amino-2,6-dimethyl-1-ethyl-3-hydroxymethyl-4,7-indoloquinone Carbamate. A suspension of 35 mg of 2,6-dimethyl-1-ethyl-3-hydroxymethyl-5-methoxy-4,7-indoloquinone carbamate (Ia)[2a] in 10 ml of methanol was treated with excess anhydrous ammonia. After 7 days the mixture was concentrated and the brown solid residue was dissolved in 3 ml of the lower phase of the system methanol–heptane, mixed with 6 g of Celite, and packed atop a column of 50 g of Celite and 25 ml of the lower phase. Elution with the upper phase gave, after concentration, 11 mg of orange solid, mp 170–171°, that was identical with starting material in infrared spectrum and did not depress the melting point of starting material. Elution with methanol afforded 23 mg of purple solid. Recrystallization of this solid from methylene chloride–hexane gave 5-amino-2,6-dimethyl-1-ethyl-3-hydroxymethyl-4,7-indoloquinone carbamate as purple granules, mp 195–205°.

Anal. Calcd for $C_{14}H_{17}N_3O_4$ (291.30): C, 57.72; H, 5.88; N, 14.43. Found: C, 57.92; H, 6.27; N, 14.27.

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Molecular Mechanisms in Nucleic Acid Photochemistry. I. Sensitized Photochemical Splitting of Thymine Dimer

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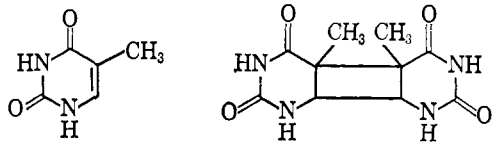
Abstract: Several compounds have been shown to be effective as sensitizers for the photochemical splitting of thymine dimer in aqueous solution. In two cases (sodium 2-triphenylenesulfonate and disodium 2,6-naphthalenedisulfonate) it has been demonstrated that the triplet state of the sensitizer is involved. Possible mechanisms are discussed including one which involves "nonvertical" triplet-energy transfer from the sensitizer to thymine dimer. Implications of these findings for the enzyme-sensitized photoreactivation of photodamaged DNA are considered.

Irradiation with ultraviolet light causes dimerization between adjacent thymines in DNA.[2] The photodimerization involves a cycloaddition of the 5,6 double bonds.[3,4] Irradiation of the thymine dimer



thymine (T) thymine dimer ($\widehat{TT}$)

($\widehat{TT}$) will lead to efficient splitting, and so a wavelength-dependent, photostationary state can be achieved.[8] The photostationary ratio lies on the dimer side for wavelengths below about 2600 Å where the dimer begins to absorb (see Figure 1).

Wacker[7] and later Setlow[9] demonstrated the presence of thymine dimer in bacteria irradiated with ultraviolet light. However, the first definitive findings involving thymine dimer in biological ultraviolet damage were those reported by the Setlows.[10] They found that transforming DNA which had been inactivated by irradiation at 2800 Å could be reactivated by subsequent irradiation at 2390 Å. Rupert, *et al.*,[11,12] found that ultraviolet-inactivated transforming DNA could be reactivated upon treatment with extracts of certain bacteria or baker's yeast in the presence of near-ultraviolet (3000–4000 Å) or even visible light. The phenomenon was shown to involve one or more enzymes. Soon afterwards, Wulff and Rupert[13] demonstrated that the thymine dimer formed in irradiated DNA *in vitro* could be eliminated by irradiating the DNA in the presence of partially purified photoreactivating enzyme extracted from baker's yeast. Later, the Setlows found an overlap between the enzyme-sensitized reactivation and the 2390-Å reversal of the lesion produced in DNA at 2800 Å. Most recently Setlow and co-workers[14] have been able to correlate the presence of thymine dimer in some synthetic polynucleotides with their ability to compete with irradiated transforming DNA for the photoreactivating enzyme. All this evidence supports the idea that thymine dimer is the major photoreactivable damage in transforming DNA and in some cells.[15]

Purpose of the Investigation. The enzyme-sensitized photoreactivation[19] has received much attention,[20,21]

(1) The Radiation Laboratory of the University of Notre Dame is operated under contract with the U. S. Atomic Energy Commission. This is AEC Document No. COO-38-432.

(2) (a) For the most current reviews see K. Smith in "Photophysiology," Vol. II, A. C. Giese, Ed., Academic Press Inc., New York, N. Y., 1964, Chapter 20; (b) A. D. McLaren and D. Shugar, "Photochemistry of Proteins and Nucleic Acids," The Macmillan Co., New York, N. Y., 1964.

(3) D. L. Wulff and G. Fraenkel, *Biochim. Biophys. Acta*, **51**, 332 (1961).

(4) There is very good evidence[5] that the dimer produced by irradiation of thymine in frozen water solution is a single stereoisomer whose configuration is the *cis-head-to-head*. It has been noted[6] that this configuration is that which would be expected for the dimer produced in DNA. There is also good evidence that the thymine dimer obtained by irradiation of thymine in ice is the same as that obtained from irradiated DNA.[7]

(5) (a) S. Y. Wang, *Photochem. Photobiol.*, **3**, 395 (1964); (b) G. M. Blackburn and R. J. H. Davies, *Chem. Commun.* (London), 215 (1965).

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(15) Recently other photoreactivable lesions have been suspected[16] and two have been identified.[17]

(16) C. S. Rupert, *Photochem. Photobiol.*, **3**, 399 (1964); J. K. Setlow, *ibid.*, **3**, 405 (1964).

(17) R. B. Setlow, W. L. Carrier, and F. J. Bollum, *Proc. Natl. Acad. Sci. U. S.*, **53**, 1111 (1965).

(18) G. N. Lewis and M. Kasha, *J. Am. Chem. Soc.*, **66**, 2108 (1944).

(19) Throughout this paper, it is assumed that the enzyme-sensitized photoreactivation involves the splitting of thymine dimers in the damaged DNA.

(20) C. S. Rupert, ref 2a, p 283, and references therein.

(21) J. Jagger, *Photochem. Photobiol.*, **3**, 451 (1964).