Journal of Medicinal Chemistry

VOLUME 10, NUMBER 1 DECEMBER 27, 1966

The Mitomycin Antibiotics. Synthetic Studies. XV.[1] The Preparation of a Related Indoloquinone[2]

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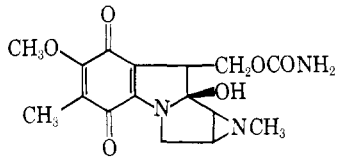
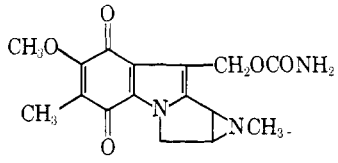
Received June 11, 1966

The synthesis and antibacterial properties of 1-ethyl-3-hydroxymethyl-5-methoxy-2,6-dimethylindole-4,7-dione carbamate (IV) are described.

In the course of their studies concerning the mitomycin antibiotics Patrick and co-workers[3] found that the related indoloquinone II retained the very potent antibacterial activity of the parent antibiotic I. We have since noted that 7-methoxymitosene (III), the deaziridino analog of II, retains in part the antibacterial properties shown by II.[4] In order to define further the structural requirements for biological effectiveness in this series, we undertook the preparation of the related 1,2-dialkylindoloquinone IV.



I



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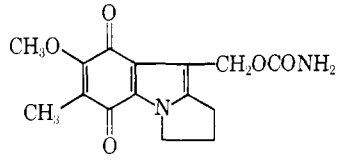
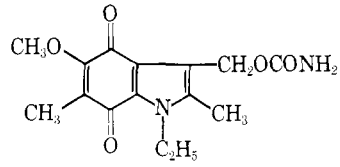


III



IV

For this synthesis, the indole ester V appeared to be a particularly suitable starting material. In principle, the development of the requisite quinone system from the 5-hydroxy group in the manner devised[4] for the synthesis of 7-methoxymitosene and the conversion[5] of the 3-ester function into a hydroxymethyl group were readily envisioned. In addition, a method was available[4] for the transformation of the last group into the carbamate ester. Ester V is readily obtained from the condensation of toluquinone with ethyl 3-ethylaminocrotonate, and its preparation is accompanied by only small amounts (<10%) of the 5-hydroxy-7-methyl isomer.[1]

For the elaboration of the 4,7-indoloquinone system, indole ester V was converted into the *o*-quinone VIa by oxidation[6] with potassium nitrosodisulfonate (Fremy's salt) (see Scheme I). Thiele acetoxylation[7] of this *o*-quinone gave hydroxyhydroquinone triacetate VIIa, which on alkaline hydrolysis and aeration of the intermediate hydroquinone solution afforded the desired 5-hydroxy-4,7-indoloquinone acid XIIa. Methylation of this hydroquinone to the required methoxyquinone XIVa (or XV) was achieved satisfactorily only after some difficulty.

Initially, the action of diazomethane on hydroxyquinone XIIa was studied, since this reagent had successfully effected the O-methylation of certain 7-hydroxymitosenes.[4,8] However, treatment of XIIa with approximately 2 equiv of diazomethane gave only 20% of methoxyquinone acid XV, accompanied by a small amount of a nonquinoid product. Three equivalents of diazomethane gave acid XV (9%) and the corresponding methyl ester XIVa (13%), as well as 10% of the nonquinoid material. After a cursory examination

(1) For paper XIV see G. R. Allen, Jr., C. Pidacks, and M. J. Weiss, *J. Am. Chem. Soc.*, **88**, 2536 (1966).

(2) A portion of this work was described in a preliminary communication: G. R. Allen, Jr., J. F. Poletto, and M. J. Weiss, *ibid.*, **86**, 3878 (1964).

(3) 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).

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

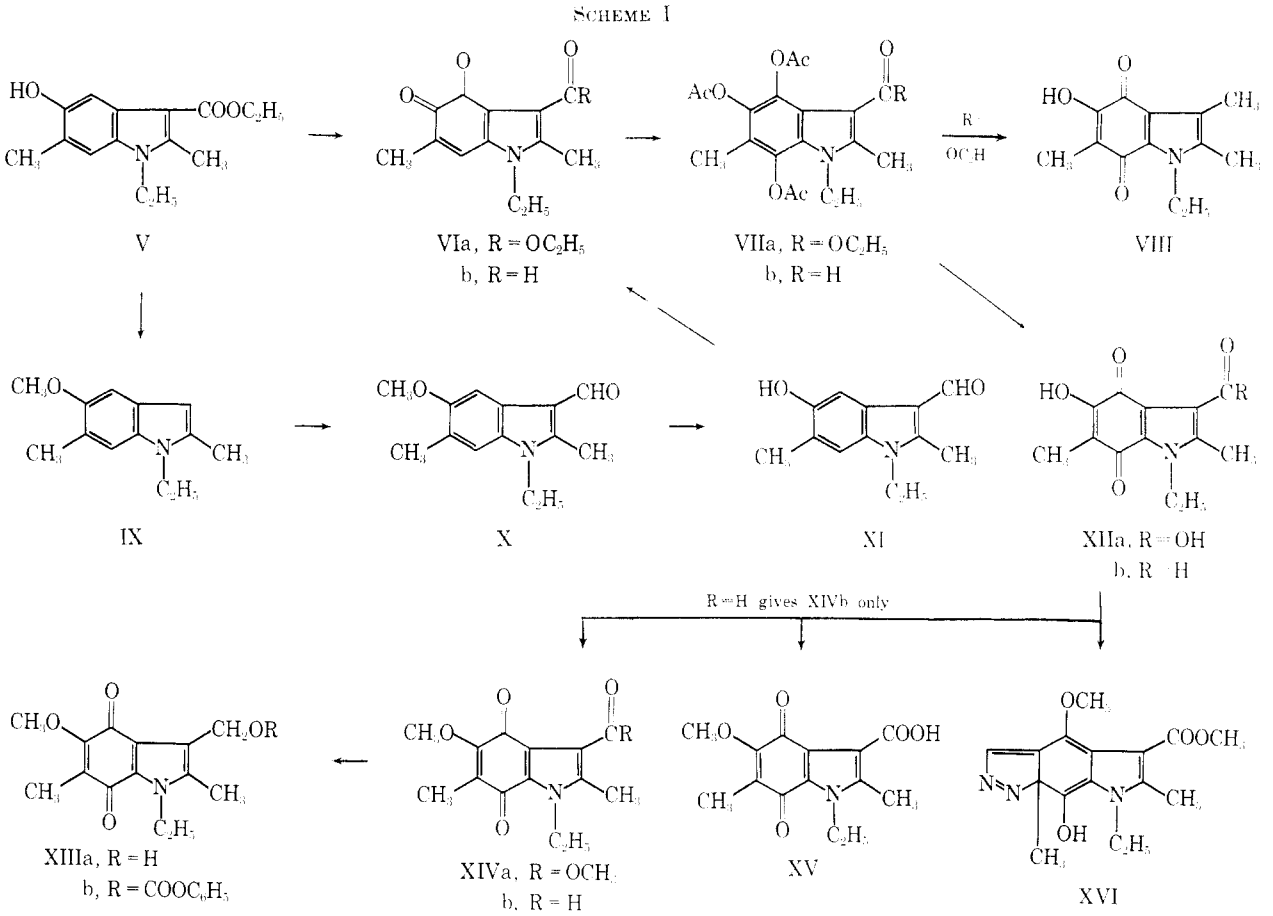
(5) (a) J. Thesing, *Ber.*, **87**, 692 (1954); (b) E. Leete, *J. Am. Chem. Soc.*, **81**, 6023 (1959).

(6) H. Teuber and G. Thaler, *Ber.*, **91**, 2253 (1958).

(7) J. Thiele, *ibid.*, **31**, 1248 (1898).

(8) (a) J. S. Webb and co-workers, unpublished work; (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).

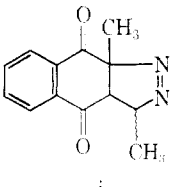
SCHEME I



of other reagents, including tetramethoxymethane,[9] it was found that methyl sulfate and potassium carbonate in acetone furnished methoxyquinone ester XIVa in good yield.

The nonquinoid product from the diazomethane experiments is best formulated as the Δ^1-pyrazole derivative XVI, resulting from addition of diazomethane to the quinone system, in light of the following facts. In the infrared spectrum of this substance there were absorption bands at 2.98 (OH), 5.85 (ester carbonyl), and 6.52 μ (N=N),[10] and conspicuous by its absence was the usual quinone carbonyl absorption in the 6-μ region. Furthermore, the ultraviolet spectrum ($\lambda_{max}^{CH_3OH}$ 320, 371 mμ; λ_{max}^{NaOH} 355, 475 mμ) was markedly different from that anticipated for an indoloquinone or indole chromophore. In addition to the resonances expected for the N-ethyl substituent, the pmr spectrum of this product was characterized by sharp singlets at 150 and 167 (3 protons each, CCH_3),[11] 236 and 243 (3 protons each, OCH_3), and 500 cps (1 proton, pyrazole H) and a broad resonance at approximately 400 cps (O*H*) which was erased on exchange with methanol-d_4. Although the 3,4 addition of diazomethane to naphthoquinone,[13] 2-substituted 1,4-naphthoquinones,[12,14] and certain benzoquinones[15] is well known, the present instance is somewhat unique inasmuch as it constitutes an example of such an addition to a tetrasubstituted quinone.[16]

Following the elaboration of the 4,7-indoloquinone system the development of the C-3 side chain was investigated. Reduction of ester XIVa with lithium aluminum hydride, followed by treatment of the reaction solution with ferric chloride, gave a complex mixture from which the desired carbinol XIIIa was isolated in only 19% yield. Treatment of naphthoquinones with lithium aluminum hydride is known to give com-



i

(9) This reagent was used successfully for the O-methylation of certain 7-hydroxymitosenes.[8a] However, treatment of hydroxyquinone acid IX with boiling tetramethoxymethane for 22 hr gave a nonquinoid product; similar treatment for 1 hr gave starting material.

(10) See T. V. Van Auken and K. L. Rinehart, Jr., *J. Am. Chem. Soc.*, **84**, 3736 (1962), and references cited therein.

(11) The chemical shift for the 2-methyl resonance in a variety of indole esters such as V is 157–163 cps,[1] whereas that for the 9a-methyl group in the isomeric 3a,4,9,9a-tetrahydro-3,9a-dimethyl-3H-benz[*f*]indazole-4,9-diones (i) is τ 8.06 (118 cps) and 8.19 (109 cps).[12] Although the chemical shift for the 8a-methyl group in the indoloquinone–diazomethane adduct XVI is significantly greater than that for the 9a-methyl group in i, we believe that this structure best accommodates the available data.

(12) F. M. Dean, P. G. Jones, R. B. Morton, and P. Sidisunthorn, *J. Chem. Soc.*, 5336 (1963).

(13) (a) L. F. Fieser and M. A. Peters, *J. Am. Chem. Soc.*, **53**, 4080 (1931); (b) F. M. Dean and P. G. Jones, *J. Chem. Soc.*, 5342 (1963).

(14) (a) L. F. Fieser and J. L. Hartwell, *J. Am. Chem. Soc.*, **57**, 1479, 1482 (1935); (b) E. Bergmann and F. Bergmann, *J. Org. Chem.*, **3**, 125 (1938).

(15) (a) F. M. Dean, P. G. Jones, and P. Sidisunthorn, *J. Chem. Soc.*, 5186 (1962); (b) F. M. Dean, P. G. Jones, R. B. Morton, and P. Sidisunthorn, *ibid.*, 411 (1964).

(16) Diazomethane reacts in a 1,2 manner with 2,3-disubstituted naphthoquinones [B. Eistert, H. Fink, and A. Müller, *Ber.*, **95**, 2403 (1962)] and tetrasubstituted benzoquinones [B. Eistert and G. Bock, *ibid.*, **92**, 1247 (1959)] to give spirooxiranes. However, the 3,4 addition of diazomethane to duroquinone was recorded recently: W. C. Howell, M. Ktenas, and J. M. MacDonald, *Tetrahedron Letters*, **No. 26**, 1719 (1964).

plex mixtures arising from 1,2 and 1,4 reduction of the quinone system,[17] and the poor yield observed in the preparation of XIIIa is possibly the result of similar side reactions. Therefore, it was conceivable that submission of a hydroquinone-3-carboxylate to lithium aluminum hydride would avoid this problem. The hydroquinone is useful in this respect since it also permits immediate regeneration of the quinone system necessary for the stabilization of the 3-hydroxymethyl function.[18] Thus, the 5-hydroxyhydroquinone-3-carboxylate triacetate VIIa was treated successively with the metal hydride and ferric chloride. Surprisingly, only the product of hydrogenolysis, the 3-methyl-*p*-quinone VIII, was isolated (63%). This observation is of interest, for hydrogenolysis of 3-hydroxymethylindoles has been reported only for those compounds lacking a nitrogen substituent; indeed, previous studies have shown that 1-alkyl-3-indolylmethanols are stable to this reagent.[5] Finally, we would note in this connection a preliminary unsuccessful attempt to reduce methoxy-*p*-quinone acid XV with diborane.

Our inability to achieve in satisfactory yield the development of the 3-hydroxymethyl group from a 3-carboxylate necessitated an alternate approach. This approach was based on that successfully used in the synthesis of 7-methoxymitosene,[4] and in this instance involved decarbethoxylation, formylation,[19] and sodium borohydride reduction. Thus, 1-ethyl-5-methoxy-2,6-dimethylindole (IX), prepared from V by decarbethoxylation[20] and O-methylation, was converted into aldehyde X by the Vilsmeier–Haack[21] technique. Transformation of this last compound into the 5-hydroxyindolealdehyde XI was accomplished with aluminum chloride in refluxing xylene. The quinone system was then introduced in the manner described above for the 3-carbethoxy series. Oxidation of XI with Fremy's salt gave *o*-quinone VIb, which on Thiele acetoxylation afforded the hydroxyhydroquinone triacetate VIIb. Alkaline hydrolysis of this last substance followed by aeration furnished the hydroxy-*p*-quinone XIIb. This quinone was transformed smoothly into the methoxy-*p*-quinone XIVb by methyl sulfate and potassium carbonate in acetone. Reduction of quinonealdehyde XIVb with sodium borohydride and subsequent oxidation of the intermediate hydroquinone with acidic ferric chloride gave the quinone alcohol XIIIa. It may be noted that the over-all yield of XIIIa from toluquinone by this ten-stage sequence was 11%. The desired indoloquinone carbamate analog IV was then obtained by ammonolysis of the phenylcarbonate XIIIb derived from alcohol XIIIa.

Biology.—The 1,2-dialkylindoloquinone IV has important antibacterial activity *in vitro* and in mice, its activity being comparable to that of 7-methoxymitosene (I). *In vitro*,[22] IV shows good activity against a spectrum of gram-positive organisms, including representative tetracycline- and penicillin-resistant strains (Table I). However, it has only marginal activity against gram-negative organisms. This analog is approximately one-third as active as tetracycline when administered orally to mice infected with *Staphylococcus aureus* var. Smith. However, IV is ineffective *in vivo* against *Streptococcus pyogenes* C203 and a tetracycline-resistant *Staphylococcus* species, despite its high activity *in vitro* against these organisms.

TABLE I

Organism	Minimum inhib concn, μg/ml[a] Indoloquinone (IV)	7-Methoxymitosene (III)	Tetracycline HCl
Mycobacterium smegmatis, ATCC 607	30	62	2
Mycobacterium ranae	30	31	2
Staphylococcus aureus, ATCC 6548P	6	8	
Staphylococcus aureus, ATCC 6538P	2	4	4
Staphylococcus aureus, 69	1	2	250
Streptococcus faecalis, ATCC 8043	8	8	4
Streptococcus pyogenes, C203	0.25	1	1
Streptococcus sp., nonhemolytic, 11	4	4	250
Streptococcus sp., β-hemolytic, 80	2	4	250
Bacillus subtilis, ATCC 6633	0.8	0.5	1
Bacillus cereus, ATCC 10702	0.25	0.5	1
Klebsiella pneumoniae, ATCC 10031	30	4	125
Pseudomonas aeruginosa, ATCC 10145	50	250	31
Proteus vulgaris, ATCC 9484	30	31	15
Escherichia coli, ATCC 9637	50	250	15
Escherichia coli, Lederle 22	30	31	2
Salmonella gallinarum, Lederle 604	50	250	15

[a] Determined in simultaneous assays.

Experimental Section

General.—Melting points were determined in an open capillary tube and are corrected. The petroleum ether used was that fraction boiling at 30–60° unless specified otherwise. Ultraviolet spectra were determined in methanol solution using a Cary recording spectrophotometer, and the infrared spectra were determined in pressed KBr disks with a Perkin-Elmer spectrophotometer (Model 21). Pmr spectra were determined with a Varian A-60 spectrometer using tetramethylsilane as an internal standard; $CDCl_3$ was used as the solvent except where noted otherwise. All evaporations were carried out at reduced pressure.

Ethyl 1-Ethyl-2,6-dimethyl-4,5-dioxo-3-indolecarboxylate (VIa).—To a mechanically stirred solution of 18.0 g (67.5 mmoles) of potassium nitrosodisulfonate in 400 ml of water and 200 ml of 0.167 *M* KH_2PO_4 was added a solution of 2.47 g (9.5 mmoles) of ethyl 1-ethyl-5-hydroxy-2,6-dimethyl-3-indolecarboxylate (V) in 500 ml of acetone. The blue color was immediately discharged, and the resulting brown solution became purple within 5 min. Stirring was discontinued; the solution was allowed to stand at room temperature for 16 hr, whereafter it was distributed between methylene chloride and water. The organic solution was dried and evaporated. The residue was slurried with ether, and the mixture was filtered to give 1.95 g (75%) of black crystals, mp 118–120°. Material from a similar experiment was recrystallized from acetone–petroleum ether (bp 60–70°) to give black crystals: mp 115–116°; λ_{max} 228, 248, 345, 540 mμ (ε 31,600, 23,300, 4720, 2600); λ 5.86, 5.99, 6.09, 7.70, 8.40, 8.60, 9.00 μ; pmr, 82 (3 protons, triplet, J = 7.5 cps, OCH_2CH_3), 85 (3 protons, triplet, J = 7.5 cps, NCH_2CH_3), 116 (3 protons, doublet, J_{CH_3H} = 2.5 cps, 6-CH_3), 147 (3 protons, 2-CH_3), 242 (2 protons, quartet,

(17) E. Boyland and D. Manson, *J. Chem. Soc.*, 1837 (1951).

(18) For a discussion of this latter point see ref 4.

(19) Following the completion of this study the direct preparation of aldehydes from carboxylic esters using sodium aluminum hydride was reported: L. I. Zakharkin, V. V. Gavrilenko, D. N. Maslin, and I. M. Khorlina, *Tetrahedron Letters*, **No. 29**, 2087 (1963). The application of this method to the present problem was not investigated.

(20) Experiment by Mr. J. F. Poletto.

(21) A. Vilsmeier and A. Haack, *Ber.*, **60**, 119 (1927).

(22) 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).

J = 7.5 cps, OCH_2CH_3), 265 (2 protons, quartet, J = 7.5 cps, NCH_2CH_3), 424 cps (1 proton, doublet, J = 2.5 cps, 7-H).

Anal. Calcd for $C_{15}H_{17}NO_4$: C, 65.44; H, 6.22; N, 5.09. Found: C, 65.41; H, 6.31; N, 5.39.

Ethyl 1-Ethyl-4,5,7-trihydroxy-2,6-dimethyl-3-indolecarboxylate Triacetate (VIIa).—A solution of 1.95 g (7.12 mmoles) of VIa (from previous experiment) in 30 ml of acetic anhydride was treated with 1 ml of boron trifluoride etherate, and the resulting brown solution was kept at room temperature for 30 min. The solution was then stirred with cracked ice until the excess acetic anhydride had hydrolyzed; considerable solid had separated. The mixture was extracted with methylene chloride, and the dried extracts were evaporated. The resulting amber gum was dissolved in ether; crystals rapidly separated to give 1.89 g (64%) of material, mp 155–158°. Material of this purity from a similar experiment was recrystallized from acetone–petroleum ether (bp 60–70°) to give white crystals: mp 157–159°; λ_{max} 222, 287 mμ (ε 23,000, 6920); λ 5.60, 5.84, 6.09, 7.50, 7.94, 8.18–8.45, 9.10, 9.30, 9.83 μ; pmr, 79 (3 protons, triplet, J = 7.5 cps, OCH_2CH_3), 83 (3 protons, triplet, J = 7.5 cps, NCH_2CH_3), 123.5 (3 protons, 6-CH_3), 141, 142.5 (9 protons, $OOCCH_3$), 157 (3 protons, 2-CH_3), 250 and 264 cps (4 protons overlapping quartets, J = 7.5 cps, OCH_2CH_3 and NCH_2CH_3, respectively).

Anal. Calcd for $C_{21}H_{23}NO_8$: C, 60.13; H, 6.01; N, 3.34. Found: C, 59.66; H, 6.22; N, 3.46.

1-Ethyl-5-hydroxy-2,6-dimethyl-4,7-dioxo-3-indolecarboxylic Acid (XIIa).—A magnetically stirred mixture of 2.373 g (5.65 mmoles) of VIIa in 100 ml of water and 20 ml of 25% NaOH solution was heated at reflux temperature under nitrogen until all solid dissolved; about 2 hr was required. The solution was filtered, and air was introduced into the filtrate for 30 min. The resulting purple solution was acidified with HCl; the resulting red-orange mixture was extracted with methylene chloride. After evaporation of the solvent, the residue was recrystallized from methylene chloride–petroleum ether to give in two crops 993 mg (67%) of red needles: mp 220–223°; λ_{max} 212, 240 (sh), 302, 335 mμ (ε 19,000, 12,800, 12,800, 5800); λ 2.93, 3.68, 5.74, 6.10, 7.40, 7.75, 8.06, 8.30, 9.30, 12.95, 13.39 μ.

Anal. Calcd for $C_{13}H_{13}NO_5$: C, 59.31; H, 4.98; N, 5.32. Found: C, 59.45; H, 5.16; N, 5.45.

Interaction of Diazomethane with XIIa.—A solution of ethereal diazomethane (prepared from 525 mg (3.6 mmoles) of N-methyl-N′-nitro-N-nitrosoguanidine) was added to a solution of 500 mg (1.9 mmoles) of XIIa in 50 ml of methylene chloride, and this solution was kept at room temperature for 2 hr. The excess diazomethane was removed in a stream of nitrogen, and the solution was evaporated. The residue was dissolved in ethyl acetate, and this solution was extracted with aqueous potassium carbamate. The dried organic solution was concentrated and chilled to give 7 mg of Δ^1-pyrazole derivative (1%, see below), mp 185–190°.

The base extract was acidified and extracted with methylene chloride. The residue obtained by evaporation of the dried solution was chromatographed on Celite (diatomaceous silica) using a heptane–ethyl acetate–methanol–water (80:20:17:4) system.[23] The material eluted at peak hold-back volume 1.4 (V_m/V_s = 3.9) was recrystallized from methylene chloride–petroleum ether to give 100 mg of 1-ethyl-5-methoxy-2,6-dimethyl-4,7-dioxo-3-indolecarboxylic acid (XV) as red-orange needles: mp 133–135°; λ_{max} 213, 240, 292, 342, 455 μ (ε 22,000, 12,700, 11,400, 3720, 620); λ 3.70, 5.76, 6.06, 6.16, 6.22, 7.56, 7.85, 10.05, 12.92 μ; pmr, 88.5 (3 protons, triplet, J = 7.5 cps, NCH_2CH_3), 128 (3 protons, 2-CH_3), 169 (3 protons, 6-CH_3), 252 (3 protons, OCH_3), 275 (2 protons, quartet, J = 7.5 cps, NCH_2CH_3), and 510 cps (broad, COOH).

Anal. Calcd for $C_{14}H_{15}NO_5$: C, 60.64; H, 5.45; N, 5.05. Found: C, 60.54; H, 5.69; N, 5.15.

Treatment of 500 mg of XIIa with the diazomethane, prepared from 900 mg (6.16 mmoles) of N-methyl-N′-nitro-N-nitrosoguanidine, for 16 hr gave an oily residue after removal of the reaction solvent. This material was dissolved in the minimum necessary quantity of ethyl acetate and chilled to give 63 mg (10%) of the Δ^1-pyrazole XVI as yellow crystals, mp 190–195°. This material was recrystallized twice from ethyl acetate–hexane to give 40 mg of pale yellow crystals: mp 197–198°; λ_{max} 258, 281, 322, 371 mμ (ε 27,100, 23,600, 6350, 5550); λ_{max}^{HCl} 258, 283, 320, 373 mμ (ε 24,600, 27,600, 6660, 5070); λ_{max}^{NaOH} 230, 255, 269, 285 (sh), 350, 475 (ε 17,800, 25,500, 25,800, 21,900, 10,300, 7940); λ 2.98, 5.82, 6.14, 6.52, 7.80, 8.23, 8.87, 9.07, 9.53, 10.15 μ; pmr (in addition to resonances cited in the discussion), 79 (3 protons, triplet, J = 7.5 cps, NCH_2CH_3) and 287 cps (2 protons, quartet, J = 7.5 cps, NCH_2CH_3).

Anal. Calcd for $C_{16}H_{19}N_3O_4$: N, 13.24. Found: N, 13.41.

The solvent was removed from the filtrate, and the residue was chromatographed on Celite using a heptane–ethyl acetate–methanol–water (85:15:17:4) system. The material eluted at peak hold-back volume 0.4 (V_m/V_s = 3.7) was recrystallized from petroleum ether to give 70 mg (13%) of methyl 1-ethyl-5-methoxy-2,6-dimethyl-4,7-dioxo-3-indolecarboxylate (XIVa) as orange needles: mp 82–83°; λ_{max} 210, 234, 286, 330, 435 mμ (ε 18,700, 14,900, 13,400, 4180, 1040); λ 5.78, 5.98, 6.10, 6.20, 7.80, 7.85, 8.75, 8.98, 9.22, 10.08 μ; pmr, 82 (3 protons, triplet, J = 7.5 cps, NCH_2CH_3), 120 (3 protons, 6-CH_3), 151 (3 protons, 2-CH_3), 240 and 248 (3 protons each, $COOCH_3$ and 5-OCH_3), and 271 cps (2 protons, quartet, J = 7.5 cps, NCH_2CH_3).

Anal. Calcd for $C_{15}H_{17}NO_5$: C, 61.85; H, 5.88; N, 4.81. Found: C, 62.16; H, 6.24; N, 4.77.

The material eluted at peak hold-back volume 1.5 was recrystallized from methylene chloride–petroleum ether to give 30 mg (9%) of the acid XV as red-orange needles, mp 133–135°.

Methyl 1-Ethyl-5-methoxy-2,6-dimethyl-4,7-dioxo-3-indolecarboxylate (XIVa).—A mixture of 681 mg (2.59 mmoles) of XIIa, 5.50 g of K_2CO_3, and 11 ml of methyl sulfate in 250 ml of acetone was stirred at reflux temperature for 45 min and then at room temperature for 2 hr. The mixture was filtered, and the salt cake was washed well with acetone. The combined filtrate and washings were evaporated, and the residue was crystallized from dilute acetone to give 490 mg (65%) of orange needles, mp 82–83°. This material was identical with the ester prepared by the diazomethane method.

1-Ethyl-3-hydroxymethyl-5-methoxy-2,6-dimethylindole-4,7-dione (XIIIa). A.—To a stirred solution of 334 mg (1.15 mmoles) of XIVa in 25 ml of tetrahydrofuran (THF) was added 197 mg of $LiAlH_4$. The resulting mixture was heated at reflux temperature for 1 hr. Ethereal $FeCl_3$ (1.0 g/20 ml) was slowly added; ethyl acetate was then added cautiously followed by water. The resulting mixture was separated, and the aqueous phase was extracted further with ethyl acetate. The combined organic solutions were washed with saline, dried, and evaporated to give 314 mg of an oil. This material was subjected to partition chromatography on Celite using a heptane–ethyl acetate–methanol–water (90:10:17:4) system. The material eluted at peak hold-back volume 3.7 (V_m/V_s = 3.9) was recrystallized from petroleum ether to give 58 mg (19%) of red-orange needles, mp 85–87°. This material was identical with that prepared by method B according to the usual criteria.

B.—A stirred solution of 500 mg (1.38 mmoles) of 1-ethyl-5-methoxy-2,6-dimethyl-4,7-dioxo-3-indolecarboxaldehyde (XIVb) in 150 ml of methanol was swept with a stream of nitrogen, heated to reflux temperature, and treated with 500 mg of $NaBH_4$. Boiling was continued for 2–3 min, and the solution was then stirred under nitrogen at room temperature for 1 hr. Acetone (5 ml) was added followed by 5 ml of a 1 N $FeCl_3$ in 0.1 N HCl solution. The resulting mixture was distributed between methylene chloride and water. The aqueous layer was extracted an additional two times with CH_2Cl_2, and the combined extracts were washed with saline, dried ($MgSO_4$), and evaporated. The residue crystallized from methylene chloride–petroleum ether to give 377 mg (75%) of red needles: mp 85.5–86.5°; λ_{max} 231, 287, 350, 460 mμ (ε 17,000, 13,900, 1300); λ 2.90–3.05, 6.00, 6.10, 6.20, 8.82, 9.08, 10.04 μ; pmr, 79 (3 protons, triplet, J = 7 cps, NCH_2CH_3), 118 (3 protons, 6-CH_3), 135 (3 protons, 2-CH_3), 240 (3 protons, 5-OCH_3), 253 (1 proton, OH, erased by exchange with D_2O), 261 (2 protons, quartet, J = 7 cps, NCH_2CH_3), 276 cps (2 protons, CH_2OH).

Anal. Calcd for $C_{14}H_{17}NO_4$: C, 63.86; H, 6.51; N, 5.32. Found: C, 63.85; H, 6.58; N, 5.41.

Yields up to 81% were obtained when this preparation was done on a 1–2-g scale.

1-Ethyl-5-hydroxy-2,3,6-trimethylindole-4,7-dione (VIII).—To a stirred solution of 419 mg (1.6 mmole) of VIIa in 25 ml of THF was added 228 mg (6.0 mmoles) of $LiAlH_4$; stirring was continued at room temperature for 1 hr. Acetic acid (4 ml) was added to the ice-cooled mixture, followed by 2-ml of a 1 N $FeCl_3$ in 0.1 N HCl solution. The mixture was distributed between CH_2Cl_2

(23) For a complete description of this technique as developed by C. Pidacks of these laboratories see M. J. Weiss, R. E. Schaub, G. R. Allen, Jr., J. F. Poletto, C. Pidacks, R. B. Conrow, and C. J. Coscia, *Tetrahedron*, **20**, 357 (1964).

and water. The organic phase was washed with water, dried, and evaporated: the residue was recrystallized from dilute methanol to give 140 mg (63%) of red needles: mp 181–182°; λ_{max} 234, 297, 363, 480 mμ (ε 16,900, 18,800, 3520, 795); λ_{max}^{HCl} 234, 299, 377, 515 mμ (ε 16,000, 17,000, 3400, 1020); λ_{max}^{NaOH} 247, 315, 363, 585 mμ (ε 19,300, 17,200, 3750, 1250); λ 3.00, 6.04, 6.15, 7.50, 8.81, 9.03, 9.55 μ; pmr, 80 (3 protons, triplet, J = 7 cps, NCH_2CH_3), 115, 131, 135 (3 protons each, 2-, 3-, and 6-CH_3), 264 (2 protons, quartet, J = 7 cps, NCH_2CH_3), and 444 cps (1 proton, OH).

Anal. Calcd for $C_{13}H_{15}NO_3$: C, 66.93; H, 6.48; N, 6.01; mol wt, 233.26. Found: C, 66.45; H, 6.37 N, 5.65; mol wt, 239, 233.

1-Ethyl-5-hydroxy-2,6-dimethylindole. A.[20]—A mixture of 20.0 g (77 mmoles) of ethyl 1-ethyl-5-hydroxy-2,6-dimethylindole-3-carboxylate (V) and 500 ml of 2 N NaOH solution was heated at reflux temperature under nitrogen for 1 hr, filtered, cooled, and acidified with HCl solution. The precipitated solid was collected and washed well with water to give 12.9 g (72%) of solid, mp 162–164° dec. A sample was recrystallized from ethyl acetate–petroleum ether (bp 60–70°) to give crystals, mp 182–183° dec, of **1-ethyl-5-hydroxy-2,6-dimethylindole-3-carboxylic acid:** λ_{max} 217, 242, 292–297 mμ (ε 33,600, 14,900, 12,300); λ 3.15 (broad), 6.05, 8.75, 910 μ.

Anal. Calcd for $C_{13}H_{15}NO_3$: C, 69.93; H, 6.48; N, 6.01. Found: 66.63; H, 6.62; N, 6.23.

The aqueous filtrate was extracted with CH_2Cl_2, and the extract was washed with water, dried (Na_2SO_4), and evaporated. The residue was recrystallized from methylene chloride–petroleum ether to give 2.92 g (20%) of crystals: mp 118–120°; λ_{max} 280, 297, 309 mμ (ε 8500, 7000, 4730); λ 2.86 μ; pmr, 76.5 (3 protons, triplet, J = 7.0 cps, NCH_2CH_3), 142 (6 protons, singlet, 2-CH_3 and 6-CH_3), 244 (2 protons, quartet, J = 7 cps, NCH_2CH_3), 284 (OH), 368 (3-H), 416 (4-H), 425 cps (low-order coupling, 7-H).

Anal. Calcd for $C_{12}H_{15}NO$: C, 76.15; H, 7.99; N, 7.90. Found: C, 75.98; H, 8.21; N, 7.51.

B.—A mixture of 175 g (0.67 mole) of VII and 1500 ml of 20% HCl solution was stirred at reflux temperature for 4 hr. A stream of nitrogen was introduced onto the surface of the mixture to reduce frothing. The cooled mixture was filtered to furnish 51.0 g of VII. The filtrate was diluted with water until the volume was about 6 l., and then extracted with ethyl acetate followed by CH_2Cl_2. The combined extracts were washed with water, dried, and evaporated to give 33 g of crude solid. The pH of the aqueous phase was then adjusted to 6–7 by addition of a concentrated KOH solution, cooled, and extracted again with CH_2Cl_2. The usual isolation procedure gave a residue which crystallized from methylene chloride–petroleum ether to give 38.1 g of crystals, mp 115–190°. The filtrate from this material was combined with the 33 g of the crude material above and passed through a Florisil magnesia–silica gel column using CH_2Cl_2 as a wash solvent. The solvent was removed from the first 3 l. of eluate, and the residue was crystallized from methylene chloride–petroleum ether to give 41.5 g of white crystals, mp 90–92°. The infrared spectra of the two crystalline modifications were identical in chloroform solution. The 51.0 g of recovered ester was recycled as described above to give 26.7 g of product; total yield, 106.3 g (83%).

In a second experiment using 170 g of VII and 1500 ml of 20% HCl solution, solution was effected after 3.5 hr at reflux temperature and 98 g (80%) of product was isolated.

1-Ethyl-5-methoxy-2,6-dimethylindole (IX).—To a stirred solution of 132 g (0.7 mole) of 1-ethyl-5-hydroxy-2,6-dimethylindole in 800 ml of ethyl alcohol and 800 ml of 4 N NaOH solution was added dropwise over 3 hr at reflux temperature under nitrogen 270 g (2.16 moles, 200 ml) of methyl sulfate. The resulting mixture was heated at reflux temperature for an additional 1.5 hr, diluted with water, and then extracted with ethyl acetate. The extract was washed with saline, dried ($MgSO_4$), and evaporated. The residual brown oil was dissolved in benzene and passed through a Florisil magnesia–silica gel column, benzene being used as the wash solvent and 500-ml fractions being collected. Fraction 1 contained 127.6 g of amber oil and fraction 2, 5.1 g (94% total). This material was of suitable purity for the next step, and partially crystallized on standing.

Material from a similar experiment was recrystallized at low temperature from hexane to give white crystals: mp 56–57°; λ_{max} 217, 278, 297, 307 mμ (ε 31,700, 8340, 7100, 4560); no hydroxyl in the infrared region; pmr, 75 (3 protons, triplet, J = 7.5 cps, NCH_2CH_3), 143, 144 (6 protons, 2-CH_3 and 6-CH_3), 232 (3 protons, OCH_3), 243 (2 protons, quartet, J = 7.5 cps, NCH_2CH_3), 371 (3-H), 422 (4-H), 426 cps (low-order coupling, 7-H).

Anal. Calcd for $C_{13}H_{17}NO$: C, 76.81; H, 8.43; N, 6.89. Found: C, 76.31; H, 8.62; N, 7.02.

1-Ethyl-5-methoxy-2,6-dimethyl-3-indolecarboxaldehyde (X).—To 200 ml of stirred, ice-chilled dimethylformamide (DMF) was added dropwise at such a rate that the temperature remained at 0–5°, 55 g (0.358 mole, 32.8 ml) of $POCl_3$. The resulting solution was treated with a solution of 66.35 g (0.326 mole) of IX, in 150 ml of DMF at such a rate that the temperature did not exceed 5°. The ice bath was removed and replaced by a warm-water bath, and the mixture was stirred at 35–40° for 1.25 hr. Cracked ice (200 g) was added, and the mixture was transferred to a 3-l. flask containing about 300 g of cracked ice, 200 ml of water being used to aid in the transfer. A solution of 250 g of NaOH in 650 ml of water was added dropwise with stirring until about one-half of the solution had been added; the remainder of the solution was added rapidly. The resulting mixture was heated to the boiling point, diluted with water to about 2.5 l., and cooled. Filtration gave 70.5 g (93%) of crystals, mp 134–136°.

Material from a preliminary experiment was recrystallized twice from acetone–hexane to give white crystals; mp 135–137°; λ_{max} 216, 258, 283, 310 mμ (ε 35,200, 18,300, 15,100, 12,500); λ 6.10 μ; pmr, 80 (3 protons, triplet, J = 7.5 cps, NCH_2CH_3), 142 (3 protons, 6-CH_3), 155 (3 protons, 2-CH_3), 238 (OCH_3), 244 (partially hidden quartet, NCH_2CH_3), 427 (broad base, 7-H), 470 (4-H), 610 cps (CHO).

Anal. Calcd for $C_{14}H_{17}NO_2$: C, 72.70; H, 7.41; N, 6.06. Found: C, 72.68; H, 7.05; N, 6.03.

1-Ethyl-5-hydroxy-2,6-dimethyl-3-indolecarboxaldehyde (XI).—A mixture of 38.4 g of 1-ethyl-5-methoxy-2,6-dimethyl-3-indolecarboxaldehyde (X) and 46.0 g of $AlCl_3$ in 1 l. of xylene was stirred at reflux temperature for 5 hr. The cooled mixture was treated with cracked ice and digested to give 38.0 g of pink solid, mp 246–250° dec. This material was of suitable purity for the subsequent reaction. Material from a pilot experiment was recrystallized from acetone to give crystals: mp 256–259° dec; λ_{max} 217, 258, 285, 311 mμ (ε 37,800, 17,600, 15,300, 15,000); λ 3.08, 6.17 μ.

Anal. Calcd for $C_{13}H_{15}NO_2$: C, 71.86; H, 6.96; N, 6.45. Found: C, 71.58; H, 7.20; N, 6.47.

1-Ethyl-2,6-dimethyl-4,5-dioxo-3-indolecarboxaldehyde (VIb).—To a stirred solution of 50.0 g (0.187 mole) of potassium nitrosodisulfonate in 1440 ml of 0.167 M KH_2PO_4 solution and 2510 ml of water was added a solution of 18.75 g (0.0864 mole) of XI in 3950 ml of hot acetone. Some solid separated, and an additional 400 ml of acetone was added. The initially blue solution turned brown on addition of the aldehyde and became purple within 5 min. Stirring was continued at room temperature for 1 hr. The reaction mixture was then concentrated, 3200 ml of distillate being collected. The concentrate was chilled and filtered. The residue was washed well with water and air-dried to give 16.05 g (80%) of black needles, mp 205–208°. A sample from a similar experiment was recrystallized from acetone–hexane to give black needles; mp 214–216°; λ_{max} 228, 285, 345, 520 mμ (ε 26,400, 6950, 2910, 1710); λ 5.97, 6.02, 6.14 μ.

Anal. Calcd for $C_{13}H_{13}NO_3$: C, 67.52; H, 5.67; N, 6.06. Found: C, 67.60; H, 5.48; N, 6.20.

1-Ethyl-4,5,7-trihydroxy-2,6-dimethyl-3-indolecarboxaldehyde Triacetate (VIIb).—To a magnetically stirred mixture of 10.00 g (43.3 mmoles) of VIb in 150 ml of acetic anhydride was added 2 ml of boron trifluoride etherate. All solid quickly dissolved, the purple mixture became brown and was stirred at room temperature for 1 hr. Cracked ice was added, and the mixture was stirred at room temperature until the excess acetic anhydride hydrolyzed. The resulting solid was collected by filtration and washed with water to give 13.3 g (82%) of gray solid, mp 184–188°. A sample from a similar experiment was recrystallized from acetone–hexane to give white crystals; mp 194–195°; λ_{max} 219, 248, 262 (shoulder), 307 mμ (ε 31,200, 18,200, 11,200, 12,400); λ 5.70, 6.05, 8.30–8.60, 9.35, 9.95 μ; pmr, 80 (3 protons, triplet, J = 7.5 cps, NCH_2CH_3), 124 (3 protons, 6-CH_3), 142.5, 145, 147.5 (9 protons, OOCCH_3), 158 (3 protons, 2-CH_3), 250 (2 protons, quartet, J = 7.5 cps, NCH_2CH_3), 613 cps (CHO).

Anal. Calcd for $C_{19}H_{21}NO_7$: C, 60.79; H, 5.64; N, 3.73. Found: C, 60.99; H, 5.70; N, 3.62.

1-Ethyl-5-hydroxy-2,6-dimethyl-4,7-dioxo-3-indolecarboxaldehyde (XIIb).—A stirred mixture of 63.5 g (0.17 mole) of VIIb in 1000 ml of water and 250 ml of 25% NaOH solution was heated

at reflux temperature under nitrogen for 1 hr. All solid dissolved and the brown solution was quickly filtered. A stream of air was introduced into the filtrate for 35 min. The resulting purple solution was acidified by addition of 37% HCl solution. Once acid, a red solid separated from the solution. It was extracted into CH_2Cl_2, and the extracts were dried ($MgSO_4$) and evaporated with concomitant addition of petroleum ether. Once crystallization began the mixture was placed in the refrigerator. Filtration gave 21.3 g of rose needles, mp 212–214°. Concentration of the filtrate gave in two crops an additional 11.0 g (78%) of product, mp 210–213°. In a preliminary experiment utilizing 750 mg of triacetate, this quinone was obtained in 89% yield as rose needles, mp 215–216°, after recrystallization from acetone–hexane; λ_{max} 220, 239, 275, 285, 298, 334, 450–465 mμ (ε 21,200, 13,300, 12,100, 12,800, 14,800, 6930, 445); λ 3.00, 3.50, 5.95, 6.11, 9.11 μ; pmr, 84 (3 protons, triplet, J = 7 cps, NCH_2CH_3), 121 (3 protons, 6-CH_3), 160.5 (3 protons, 2-CH_3), 272 (2 protons, quartet, J = 7 cps, NCH_2CH_3), 441 (1 proton, OH), 638 cps (CHO).

Anal. Calcd for $C_{13}H_{13}NO_4$: C, 63.15; H, 5.30; N, 5.67. Found: C, 63.38; H, 5.47; N, 5.66.

1-Ethyl-5-methoxy-2,6-dimethyl-4,7-dioxo-3-indolecarboxaldehyde (XIVb).—A stirred mixture of 21.9 g (0.089 mole) of XIIb, 100 g of K_2CO_3, and 67.5 g (0.535 mole, 50 ml) of dimethyl sulfate in 1 l. of acetone was heated at reflux temperature for 1 hr, whereafter stirring was continued at room temperature for 3 hr. The mixture was filtered, and the residue was washed well with acetone. The combined filtrate and washings were evaporated, the excess dimethyl sulfate being removed at oil-pump pressure. The residue was dissolved in 200 ml of acetone and treated, with stirring, with about 800 ml of water. Stirring was continued for 30 min, and the resulting mixture was chilled and filtered to furnish 18.9 g of needles, mp 124–127°. For purification this material was dissolved in CH_2Cl_2 and passed through a Florisil column, methylene chloride being used as a wash solvent. The eluate was essentially colorless after 2.5 l. was collected. The solvent was removed, and the residue was crystallized from methylene chloride–petroleum ether to give 15.08 g (65% yield) of red needles: mp 133–135°; λ_{max} 218, 245, 270, 280 (sh), 330, 430 mμ (ε 18,300, 11,000, 11,400, 10,500, 4460, 759); λ 3.50, 5.95, 6.07, 6.18, 9.05, 10.00 μ; pmr, 81 (3 protons, triplet, J = 7 cps, NCH_2CH_3), 118 (3 protons, 6-CH_3), 157 (3 protons, 2-CH_3), 243 (3 protons, OCH_3), 265 (2 protons, quartet, J = 7 cps, NCH_2CH_3), 630 cps (CHO).

Anal. Calcd for $C_{14}H_{15}NO_4$: C, 64.36; H, 5.79; N, 5.36. Found: C, 63.96; H, 5.71; N, 5.65.

In a pilot experiment this quinone was obtained in 91% yield and experiments conducted with 2–5 g of hydroxyquinone gave 78–84% of product.

1-Ethyl-3-hydroxymethyl-5-methoxy-2,6-dimethylindole-4,7-dione Phenylcarbonate (XIIIb).—To an ice-chilled, stirred solution of 3.00 g (11.4 mmoles) of 1-ethyl-3-hydroxymethyl-5-methoxy-2,6-dimethylindole-4,7-dione (XIIIa) in 30 ml of pyridine was added dropwise 3.1 ml of phenyl chloroformate. The resulting mixture was stirred at room temperature for 2.5 hr and then diluted with water to give 4.30 g (98%) of orange needles, mp 107–111°. A sample from a similar experiment was recrystallized from methylene chloride–petroleum ether to give orange needles: mp 115–117°; λ_{max} 231, 285, 345, 455 mμ (ε 17,700, 13,800, 3260, 1150); λ 5.68, 6.00, 6.07, 6.19, 7.90–8.10, 9.00, 14.53 μ; pmr, 80 (3 protons, triplet, J = 7 cps, NCH_2CH_3), 118 (3 protons, 6-CH_3), 140 (3 protons, 2-CH_3), 242 (3 protons, OCH_3), 262 (2 protons, quartet, J = 7 cps, NCH_2CH_3), 323 (2 protons, CH_2O), 436 and 439 cps (aryl protons).

Anal. Calcd for $C_{21}H_{21}NO_6$: C, 65.78; H, 5.52; N, 3.65. Found: C, 66.01; H, 5.71; N, 3.72.

1-Ethyl-3-hydroxymethyl-5-methoxy-2,6-dimethylindole-4,7-dione Carbamate (IV).—To 50 ml of liquid NH_3 was added dropwise with stirring a solution of 2.00 g (5.22 mmoles) of 1-ethyl-3-hydroxymethyl-5-methoxy-2,6-dimethylindole-4,7-dione phenylcarbonate (XIIIb) in 100 ml of CH_2Cl_2. The resulting solution was stirred at room temperature for 2 hr during which time most of the ammonia evaporated. A warm-water bath was placed under the reaction vessel, and a stream of nitrogen was introduced until the ammonia had completely evaporated. The concentrate was dried ($MgSO_4$) and evaporated with concomitant addition of petroleum ether until crystallization began. This gave 1.323 g (83%) of orange crystals: mp 202–204°; λ_{max} 231, 286, 345, 455 mμ (ε 18,400, 13,800, 3520, 1290); λ 2.90, 3.01, 3.06, 5.80, 6.09, 6.25, 6.60, 9.05, 9.78 μ; pmr (in deuteriodimethyl sulfoxide), 74 (3 protons, triplet, J = 7 cps, NCH_2CH_3), 112 (3 protons, 6-CH_3), 138 (3 protons, 2-CH_3), 235 (3 protons, OCH_3), 260 (2 protons, quartet, J = 7 cps, NCH_2CH_3), 305 (2 protons, CH_2O), 387 cps ($CONH_2$).

Anal. Calcd for $C_{15}H_{18}N_2O_5$: C, 58.81; H, 5.92; N, 9.15. Found: C, 58.38; H, 6.07; N, 9.22.

Acknowledgment.—Microanalyses were furnished by Mr. L. Brancone and his staff, and spectral data by Mr. W. Fulmor and his associates. The partition chromatography was performed by Mr. C. Pidacks and his staff. *In vitro* antibacterial assays were performed by Mr. A. C. Dornbush and his group, and *in vivo* antibacterial data were furnished by Mr. G. S. Redin and his associates.