Synthesis of the Bacterial Coenzyme Methoxatin

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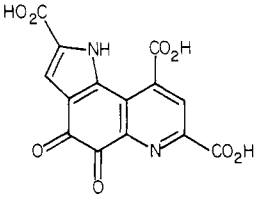
The details of a total synthesis of methoxatin (1), the coenzyme of several bacterial alcohol dehydrogenases, are presented. Methoxatin has been prepared in 13 steps by starting from 2,3-dimethoxytoluene. The successful synthetic strategy for construction of the coenzyme used a Pfitzinger synthesis for preparation of the quinoline dicarboxylic acid portion of 1 and an "umpolung" variation of the Reissert indole synthesis for annulation of the remaining pyrrole ring.

About 20 years ago, Anthony and Zatman[1] discovered that a *Pseudomonas* species which was capable of growth using methanol as its exclusive source of energy and cellular carbon[2] contained an alcohol dehydrogenase which rapidly oxidized formaldehyde and methanol. This enzyme was unusual in that it was NAD independent, did not react directly with oxygen, and contained no iron or heme. Additionally, it was found that the enzyme was not a flavoprotein and that either ammonia or a primary amine was required as an activator. Several other types of methylotrophic bacteria[2] were subsequently found to contain methanol dehydrogenases with similar properties.[3] It was recognized in 1967 that methanol dehydrogenase contains a unique prosthetic group,[1] but only recently has the chemical nature of this species been elucidated.[4,5]

In 1979 Forrest et al.[5] suggested structure 1 for the coenzyme (to which they assigned the trivial name methoxatin) on the basis of an X-ray crystallographic study on the derivative 2. Compound 2 was believed to have been



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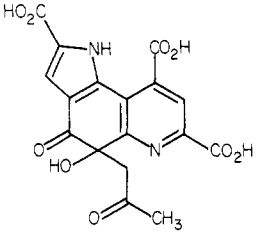

2

formed by condensation of the coenzyme with acetone during the purification process. Since methoxatin was previously suggested to be an orthoquinone,[6] structure 1 indeed seemed logical for the coenzyme. Support for this structure was recently provided via spectral studies performed by Duine, Frank, et al.[4]

It appears that methoxatin is a prosthetic group in some alcohol dehydrogenases other than those found in methylotrophs. For example, *Acinetobacter calcoaceticus*, which cannot subsist on methanol, contains a dehydrogenase which allows it to metabolize ethanol and higher alcohols. This enzyme, although quite different from the methanol dehydrogenases, contains methoxatin.[7] Also, glucose dehydrogenase from this same organism seems to have methoxatin as its coenzyme.[8] At present, it is not clear just how widespread methoxatin-based enzymes may be. Interest in methylotrophic bacteria has been heightened by the fact that these organisms are used in a commercial method for production of single-cell proteins from methanol.[9]

Several important questions concerning methoxatin remain to be answered, not the least of which concerns its mechanism of action as an alcohol oxidant.[4a,5b,10] It would also be of interest to know the biosynthetic origin of the coenzyme. Another problem as yet unsolved has been to develop a method to reconstitute methanol dehydrogenase from the apoenzyme and coenzyme. To date, only very small quantities of methoxatin have been isolated from natural sources, certainly not enough to provide adequate answers to the aforementioned questions.

We recently reported an efficient total synthesis of methoxatin[11] which confirms the proposed structure and which makes significant quantities of the cofactor available for a variety of biological studies. Corey and Tramontano have independently developed a total synthesis of methoxatin using an approach different from ours.[12] In this paper we describe the complete details of our work.

Our initial synthetic strategy for construction of 1 was to first prepare a suitable quinolinedicarboxylic acid ring system via a Pfitzinger synthesis and then to attach the remaining ring through a Reissert indole synthesis. As it turned out, the former annulation proved to be straightforward, and the latter did not.

The starting material for the synthesis was 2,3-dimethoxytoluene (3) which was metalated[13] with butyllithium/TMEDA in hexane at ~20 °C. The anion was then quenched with CO_2 at −78 °C to afford the desired benzoic acid 4[14] (66%) along with a very small amount (<10%) of phenylacetic acid 5 (eq 1).

Acid 4 was elaborated into the requisite quinolinedicarboxylic acid system as outlined in Scheme I. Curtius rearrangement of 4 afforded the previously unknown aniline 6 in 65% yield. A Sandmeyer isatin synthesis was then used to convert 6 to 8. Treatment of aniline 6 with chloral hydrate, hydroxylamine hydrochloride, and sodium sulfate in water yielded crystalline oximino compound 7

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(2) Colby, J.; Dalton, H.; Whittenbury, R. *Annu. Rev. Microbiol.* **1979**, *33*, 481.
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(4) (a) Duine, J. A.; Frank, J. *Biochem. J.* **1980**, *187*, 213. (b) Duine, J. A.; Frank, J.; Verwiel, P. E. J. *Eur. J. Biochem.* **1980**, *108*, 187. (c) Duine, J. A.; Frank, J. *Biochem. J.* **1980**, *187*, 221. Duine, J. A.; Frank, J.; Verwiel, P. E. J. *Eur J. Biochem.* **1981**, *118*, 395.
(5) (a) Salisbury, S. A.; Forrest, H. S.; Cruse, W. B. T.; Kennard, O. *Nature (London)* **1979**, *280*, 843. (b) Forrest, H. S.; Salisbury, S. A.; Kilty, C. G. *Biochem. Biophys. Res. Commun.* **1980**, *97*, 248. (c) Forrest, H. S.; Salisbury, S. A.; Sperl, G. *Biochem. Biophys. Acta* **1981**, *676*, 226.
(6) Westerling, J.; Frank, J.; Duine, J. A. *Biochem. Biophys. Res. Commun.* **1979**, *87*, 719.
(7) Duine, J. A.; Frank, J. *J. Gen. Microbiol.* **1981**, *122*, 201.
(8) Duine, J. A.; Frank, J.; Van Zeeland, J. K. *FEBS Lett.* **1979**, *108*, 443.
(9) Windass, J. D.; Worsey, M. J.; Pioli, E. M.; Pioli, D.; Barth, P. T.; Atherton, K. T.; Dart, E. C.; Byrom, D.; Powell, K.; Senior, P. J. *Nature (London)* **1980**, *287*, 396 and references cited therein.
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(12) Corey, E. J.; Tramontano, A. *J. Am. Chem. Soc.* **1981**, *103*, 5599.
(13) Gschwend, H. W.; Rodriquez, H. R. *Org. React.* **1979**, *26*, 1.
(14) For a previous low-yield synthesis of 3 see: Lovie, J. C.; Thomson, R. H. *J. Chem. Soc.* **1961**, 485.

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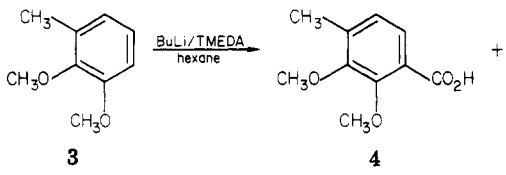
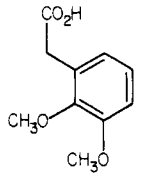


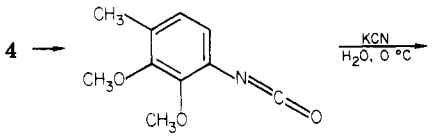
3 4



5 (1)

(73%).[15] Without purification, 7 was heated with polyphosphoric acid at 100 °C to give orange isatin 8 (70%).

A potentially more efficient route for conversion of 4 to 8 was investigated at this point. Isocyanate 11, which is an intermediate in the conversion of acid 4 to aniline 6, was easily isolated and was treated with potassium cyanide to afford acyl nitrile 12 (eq 2). However, exposure of 12



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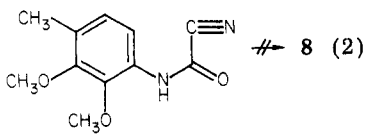


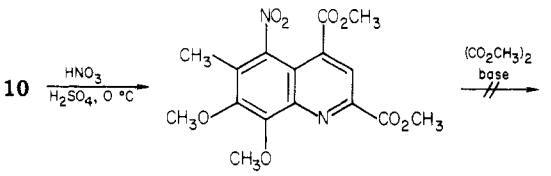
12

to hot polyphosphoric acid under conditions previously used to transform oxime 7 to isatin 8 failed to produce any 8.[16] Similarly, treatment of 12 with anhydrous aluminum chloride gave no isatin. These experiments would seem to indicate that nitrile 12 is *not* an intermediate in cyclization of 7 to 8. However, one can envision other mechanistic routes for this ring closure not involving a nitrile.

Application of the Pfitzinger quinoline synthesis to isatin 8 by using pyruvic acid and KOH afforded diacid 9.[17] This compound was not purified but was immediately converted to the dimethyl ester 10 (50% from 8).

Our next goal was to annulate the remaining pyrrole ring onto quinoline 10, and it was originally our intention to do this via a classical Reissert indole synthesis.[18] Thus, compound 10 was nitrated to give 13 in 60% yield (eq 3). However, all attempts to condense 13 with dimethyl oxalate by using a variety of alkoxide bases failed, and the desired α-keto ester 14 could not be detected. An attempt was also made to deprotonate 13 with LDA, but a subsequent D_2O quench indicated that the desired carbanion had not formed. It is possible that crowding in 13 forces the nitro group out of the plane of the aromatic ring and thus precludes resonance stabilization of the benzyl anion.

Since the obvious approach to synthesis of keto ester 14 could not be executed due to our inability to form the



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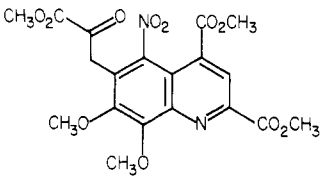
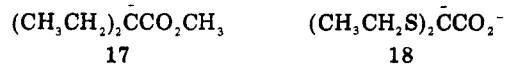


14 (3)

appropriate carbanion, we decided to investigate an "umpolung" synthesis of this compound. Attempts at benzylic bromination of 13 to produce 15 failed, but compound 10 was cleanly (100%) converted to bromide 16 with NBS (Scheme II). Low-temperature nitration of 16 afforded the desired intermediate 15 (65%).

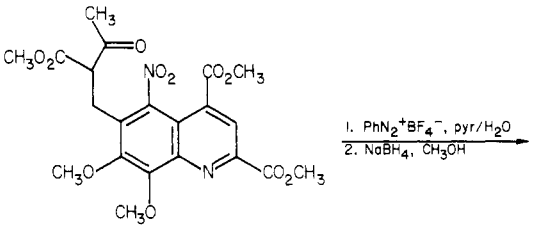
A number of unsuccessful attempts were made to condense bromide 15 with some oxalate acyl carbanion equivalents such as 17[19] and 18.[20] Similarly, bromide 16



17 18

did not alkylate either of these carbanions. However, the sodium enolate of methyl acetoacetate nicely combined with bromide 15 to afford keto ester 19 (91%).

Degradation of 19 to the desired "Reissert intermediate" 20 (eq 4) was effected using Kozikowski's modification[21]



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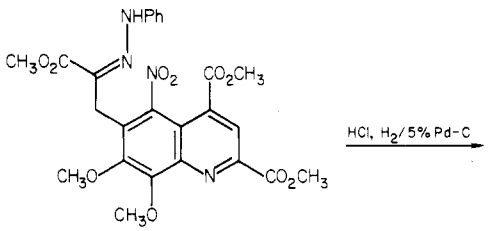

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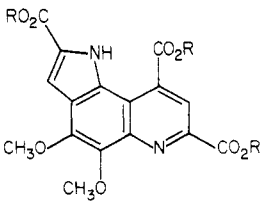
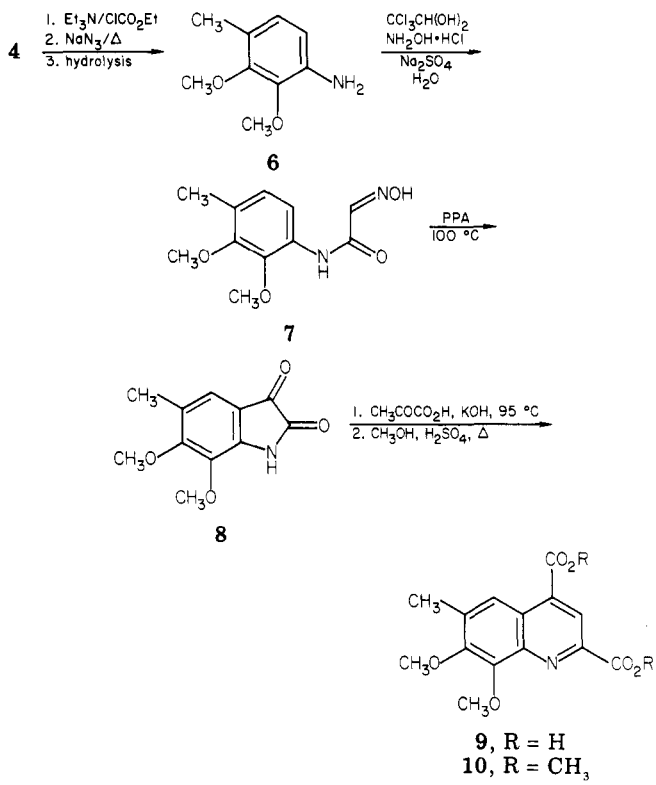


21, R = CH_3
22, R = H (4)

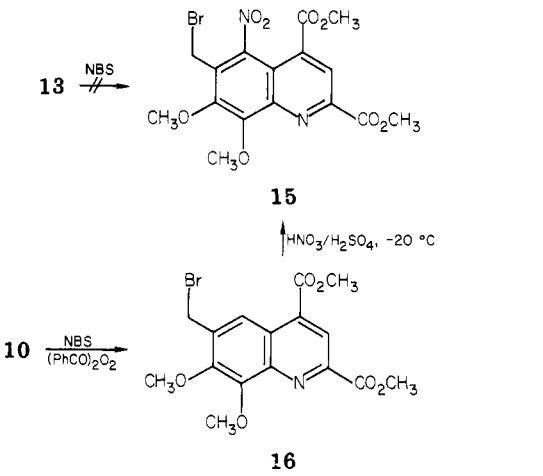
of the Japp–Klingemann reaction.[22] Thus, treatment of 19 with benzenediazonium fluoroborate in aqueous pyri-

(15) Karnes, H. A.; Wilson, M. H.; Margrave, J. L.; Newman, M. S. *J. Am. Chem. Soc.* **1965**, *87*, 5554.
(16) An isatin synthesis of this type has been reported: Kranzlein, G.; Wolfram, A.; Hausdörfer, E. U.S. Patent 1 792 170; *Chem. Abstr.* **1931**, *25*, 1845.
(17) Senear, A. E.; Sargent, H.; Mead, J. F.; Koepfli, J. B. *J. Am. Chem. Soc.* **1946**, *68*, 2695.
(18) Noland, W. E.; Baude, F. J. "Organic Syntheses"; Wiley: New York, 1973; Collect. Vol. V, p 567.
(19) Lerner, L. M. *J. Org. Chem.* **1976**, *41*, 2228.
(20) Bates, G. S. *J. Chem. Soc., Chem. Commun.* **1979**, 161.
(21) Kozikowski, A. P.; Floyd, W. C. *Tetrahedron Lett.* **1978**, 19.
(22) Phillips, R. R. *Org. React.* **1959**, *10*, 143.

Scheme I



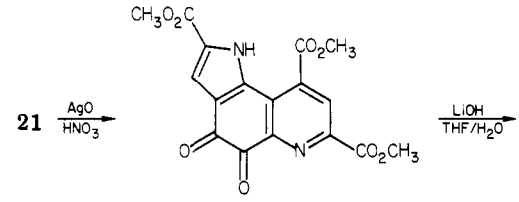
Scheme II



dine, followed by reduction and fragmentation of the intermediate diazo compound[22] with sodium borohydride, afforded a 70% yield of yellow crystalline hydrazone **20**.

To our delight, catalytic hydrogenation of the nitro group of **20** led *directly* to tricyclic triester **21** (66%). The final stages of the total synthesis only required ester cleavage and oxidation of the central carbocyclic ring of **21** to the corresponding *o*-quinone. These steps proved somewhat more difficult than initially envisioned.

Triester **21** could be hydrolyzed to the triacid **22** with methanolic KOH, but this compound could not be oxidized to the desired quinone. Triester **21**, however, was readily oxidized by AgO[23] to quinone **23** (eq 5). A hydrolysis procedure for converting **23** to methoxatin proved extremely difficult to develop due to the sensitivity of these compounds to basic reagents. Treatment of **23** with KOH, NaOH, K_2CO_3, or NH_4OH under various conditions led to its destruction. However, we eventually discovered that



hydrolysis of **23** to methoxatin (1) could be cleanly effected (75%) with lithium hydroxide in aqueous THF at room temperature. Our synthetic coenzyme had UV and ^1H NMR spectra as reported for the natural compound.[4] In order to firmly establish identity with natural material, we treated synthetic 1 with acetone containing some ammonium hydroxide, affording adduct **2** which was identical in ^1H NMR, UV, and TLC with an authentic sample.

Thus, we have developed a total synthesis of methoxatin in 13 steps starting from 2,3-dimethoxytoluene. Material prepared by our synthesis has been used in electrochemical studies aimed at elucidating the role of the coenzyme in alcohol oxidations.[10b]

Experimental Section

General Methods. All nonaqueous reactions were run under a positive pressure of dry nitrogen. Melting points were obtained in open capillary tubes on a Thomas-Hoover apparatus and are uncorrected. Infrared spectra (IR) were recorded on a Perkin-Elmer Model 197 spectrometer. ^1H nuclear magnetic resonance spectra (^1H NMR) were obtained in the solvents indicated at 60 MHz on a Varian EM-360 NMR spectrometer, at 200 MHz on a Bruker WP 200 spectrometer, and at 360 MHz on a Bruker WM 360 spectrometer. Chemical shifts are reported in δ units (parts per million) downfield of tetramethylsilane. Splitting patterns are designated as follows: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet. Carbon-13 nuclear magnet resonance spectra (^{13}C NMR) were obtained on a Varian CFT-20 NMR spectrometer, and chemical shifts are recorded in δ units downfield of tetramethylsilane. Elemental analyses were performed by Micro Tech Laboratories, Inc., Skokie, IL. Low-resolution and high-resolution mass spectra were obtained at 50–70 eV by electron impact (EI) on a Kratos MS9/50 double-focusing mass spectrometer. Ultraviolet–visible absorption spectra (UV) were recorded on either a Cary 17 or Cary 118 C spectrometer. Both analytical and preparative thin-layer chromatographies were performed by using E. M. Merck silica gel 60 PF-254, and column chromatography was done by using 70–230-mesh silica gel 60 (E. M. Merck) as the stationary phase. Reverse-phase chromatography was done on Waters Associates Seppak C_{18} cartridges.

2,3-Dimethoxy-4-methylbenzoic Acid (4). To a mechanically stirred solution of 400 mL of dry hexane containing 86 mL of 1.5 M *n*-butyllithium (0.13 mol) and 20 mL (15.4 g, 0.13 mol) of TMEDA was added dropwise 17.2 mL (17.8 g, 0.117 mol) of 2,3-dimethoxytoluene (**3**). A bright yellow precipitate formed. The mixture was stirred for 18 h at room temperature, cooled to −78 °C, and saturated with dry CO_2 gas. After 1 h, 300 mL of water was added. The aqueous solution was acidified with concentrated HCl and extracted with ethyl acetate, and the organic layer was washed with saturated $NaHCO_3$. The aqueous layer was separated, acidified, and extracted with ethyl acetate. The organic extract was washed with brine, dried (Na_2SO_4), and concentrated in vacuo to produce 15.1 g (66%) of crude solid acid 4 sufficiently pure for use in the next step. A sample was recrystallized from hexane: mp 121–122 °C (lit.[14] mp 125 °C); IR ($CHCl_3$) 3850, 1735, 1605, 1260 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.33 (3 H, s), 3.83 (3 H, s), 4.08 (3 H, s), 6.98 (1 H, d, J = 8 Hz), 7.70 (1 H, d, J = 8 Hz), 11.2 (1 H, br s).

2,3-Dimethoxy-4-methylbenzenamine (6). A solution of 4.9 mL (3.6 g, 36 mmol) of triethylamine in 35 mL of acetone was added dropwise to an ice-cold solution of 5.8 g (30 mmol) of acid 4 in 70 mL of acetone. To the resulting solution was added dropwise a solution of 3.9 mL (4.4 g, 41 mmol) of ethyl chloro-

(23) Snyder, C. D.; Rapoport, H. *J. Am. Chem. Soc.* **1972**, *94*, 227.

formate in 35 mL of acetone. The mixture was stirred at 0 °C for 1 h, and a solution of 3.1 g (48 mmol) of sodium azide in 8 mL of water was added dropwise. The resulting mixture was stirred for 1 h at 0 °C and was diluted with ice–water, and the solution was extracted with toluene. The organic layer was washed with brine and dried (Na_2SO_4). The solution of acyl azide in toluene was gently heated, and N_2 evolution was observed. The solution was concentrated in vacuo to a volume of 50 mL, and 20 mL of 50% aqueous KOH was added. The two-phase system was heated gently until no isocyanate was detectable by TLC. The reaction mixture was cooled, carefully acidified with 20% aqueous HCl, and extracted with ether. The aqueous layer was separated, neutralized with $NaHCO_3$, and extracted with ether. The organic extract was dried ($MgSO_4$) and concentrated in vacuo. Purification of the crude product was accomplished by sublimation (0.1 torr, 40 °C), producing 3.2 g (65%) of the aniline **6** as a white solid: mp 45–46 °C; IR (film) 3460, 3365, 1610 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.13 (3 H, s), 3.78 (3 H, s), 3.81 (3 H, s), 6.33 (1 H, d, J = 8 Hz), 6.65 (1 H, br d, J = 8 Hz); ^{13}C NMR ($CDCl_3$) δ 15.08, 59.67, 59.86, 110.69, 121.04, 125.28, 138.67, 140.37, 151.46.

***N*-(2,3-Dimethoxy-4-methylphenyl)-2-(hydroxyimino)-acetamide (7).** A solution of 1.517 g (9.1 mmol) of aniline **6** in 7 mL of H_2O containing 0.75 mL of concentrated HCl was added to a solution of 1.68 g (10.2 mmol) of chloral hydrate, 2.03 g (29.2 mmol) of hydroxylamine hydrochloride, and 13.4 g of anhydrous Na_2SO_4 in 45 mL of water which had previously been stirred at 45–50 °C for 15 min. The resulting mixture was heated at 45–50 °C for 1 h, 55–60 °C for 1 h, and 65–70 °C for 1 h. An additional 0.45 g of chloral hydrate and 4.1 g of hydroxylamine hydrochloride were added, and the reaction mixture was heated at 75–80 °C for 1 h. When the mixture was cooled in ice–water, a light brown precipitate formed which on filtration produced 1.58 g (73%) of crude **7** of sufficient purity for use in the next step.

6,7-Dimethoxy-5-methyl-1*H*-indole-2,3-dione (8). To 20 mL of polyphosphoric acid at 100 °C was added 2.00 g (8.39 mmol) of **7** portionwise over a period of 5 min. After 20 min the reaction mixture was cooled, and 40 mL of water was added carefully. An orange precipitate formed which was collected by filtration, yielding 1.30 g (70%) of isatin **8**. A sample was recrystallized from methanol: mp 180.5–181.5 °C; IR (KBr) 3180, 1755, 1735 cm^{-1}; ^1H NMR (acetone-d_6) δ 2.17 (3 H, s), 3.87 (3 H, s), 3.98 (3 H, s) 7.15 (1 H, br s), 10.03 (1 H, br); ^{13}C NMR (Me_2SO-d_6) δ 15.34, 60.31, 60.94, 113.42, 122.16, 125.34, 137.10, 143.65, 159.37, 159.10, 182.51. Anal. Calcd for $C_{11}H_{11}NO_4$: C, 59.73; H, 5.01; N, 6.33. Found: C, 59.97; H, 5.05; N, 6.22.

Dimethyl 7,8-Dimethoxy-6-methyl-2,4-quinolinedicarboxylate (10). A suspension of 1.20 g (5.42 mmol) of isatin **8** in 33 mL of 30% KOH initially was heated to 95 °C. When solution was obtained, 3.7 mL of 33% (w/v) of aqueous pyruvic acid was added dropwise. The resulting solution was stirred at 95 °C for 6 h. A precipitate was observed upon cooling of the solution, and the mixture was cooled during neutralization with concentrated HCl. The aqueous solution was extracted with ethyl acetate, and the organic layer was extracted with saturated $NaHCO_3$. The aqueous layer was carefully acidified with concentrated HCl, producing a very fine, bright yellow solid. Water was removed in vacuo, and the solid residue was treated with 50 mL of 10% methanolic H_2SO_4. The mixture was heated at reflux for 4 h and was cooled. Water was added, and the product was extracted with ethyl acetate. The organic layer was washed with saturated $NaHCO_3$ and brine and dried (Na_2SO_4). Evaporation in vacuo followed by recrystallization of the residue from ethyl acetate afforded 0.87 g (50%) of quinoline diester **10** as fluorescent yellow crystals: mp 133–134 °C; IR (KBr) 1725, 1615, 1255 cm^{-1}; ^1H NMR (CCl_4) δ 2.43 (3 H, br s), 3.98 (6 H, s), 4.05 (3 H, s), 4.16 (3 H, s), 8.27 (1 H, br s), 8.38 (1 H, s); mass spectrum, m/e (relative intensity) 319 (26.5), 304 (100), 288 (9.4), 258 (58.9); exact mass calcd for $C_{16}H_{17}NO_6$ 319.1056, found 319.1061.

Dimethyl 7,8-Dimethoxy-6-methyl-5-nitro-2,4-quinolinedicarboxylate (13). To an ice-cold solution of 110 mg of quinoline diester **10** in 2.5 mL of concentrated H_2SO_4 was rapidly added 2.0 mL of a 1/1 mixture of concentrated H_2SO_4/concentrated HNO_3. The mixture was rapidly stirred at 0 °C for 1.5 min, and 30 mL of ice–water was added. The mixture was neutralized with saturated $NaHCO_3$ and extracted with ethyl acetate. The organic layer was washed with brine and dried ($NaSO_4$). The yellow-green oil resulting on concentration of the extract in vacuo was chromatographed on a column of silica gel (6 g), eluting with ethyl acetate–hexane (2:3) to afford 75 mg (60%) of the nitrated quinoline **13** as a light yellow-green solid: mp 116–117 °C; IR (film) 1730, 1525, 1350 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.52 (3 H, s), 3.80 (3 H, s), 3.96 (3 H, s), 4.11 (3 H, s), 4.32 (3 H, s), 8.40 (1 H, s); mass spectrum, m/e (relative intensity) 364 (14.0), 363 (11.9), 349 (100), 318 (14), 288 (38.3).

Dimethyl 6-(Bromomethyl)-7,8-dimethoxy-2,4-quinolinedicarboxylate (16). A mixture of 1.495 g (4.682 mmol) of quinoline **10**, 0.891 g (5.00 mmol) of *N*-bromosuccinimide, and a catalytic amount of benzoyl peroxide in 60 mL of CCl_4 was refluxed for 3 h. The solvent was removed in vacuo, and the solid residue was dissolved in ethyl acetate. The organic layer was washed successively with saturated $NaHSO_3$, saturated $NaHCO_3$, and brine. The solution was dried (Na_2SO_4) and concentrated in vacuo to yield 1.86 g (100%) of bromide **16** of sufficient purity for use in the next step. A sample was recrystallized from ethyl acetate: mp 181.5–182.5 °C; IR (KBr) 1715, 1610, 1230 cm^{-1}; ^1H NMR ($CDCl_3$) δ 4.03 (6 H, s), 4.18 (6 H, s), 4.66 (2 H, s), 8.56 (1 H, s), 8.60 (1 H, s); mass spectrum, m/e (relative intensity) 384 (93.2), 382 (99.4), 318 (100), 304 (64.9), 59 (28.5). Anal. Calcd for $C_{15}H_{16}BrNO_6$: C, 43.26; H, 4.05; N, 3.53. Found: C, 48.23; H, 4.02; N, 3.49.

Dimethyl 6-(Bromomethyl)-7,8-dimethoxy-5-nitro-2,4-quinolinedicarboxylate (15). To a solution of 0.359 g (0.900 mmol) of bromide **16** in 4.0 mL of concentrated H_2SO_4 at −20 °C was added rapidly 3.0 mL of a 1/1 mixture of concentrated H_2SO_4/concentrated HNO_3. The mixture was stirred rapidly for 2.5 min and was quenched by addition of ice–water. The mixture was neutralized with saturated $NaHCO_3$ and was extracted with ethyl acetate. The organic layer was washed with water and brine and dried (Na_2SO_4). Concentration of the solution in vacuo followed by chromatography of the residue on 18 g of silica gel, eluting with ethyl acetate–hexane (1:2), yielded 0.259 g (65%) of pure nitrated bromide **15**. A sample was recrystallized from ethyl acetate/hexane: mp 114–115 °C; IR (KBr) 1730, 1600, 1530, 1355, 1270 cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.90 (3 H, s), 4.03 (3 H, s), 4.18 (3 H, s), 4.27 (3 H, s), 4.71 (2 H, s), 8.38 (1 H, s); ^{13}C NMR ($CDCl_3$) δ 20.91, 52.81, 53.30, 62.23, 63.15, 115.67, 122.21, 122.59, 131.16, 137.83, 142.94, 147.84, 149.53, 150.71, 164.17, 165.28; mass spectrum, m/e (relative intensity) 444 (8.4), 442 (8.4), 429 (82.5), 427 (82), 398 (11), 396 (11.6), 363 (28.8), 349 (88.4), 318 (27.1), 303 (50.4), 302 (50.3).

Dimethyl 7,8-Dimethoxy-6-[2-(methoxycarbonyl)-3-oxobutyl]-5-nitro-2,4-quinolinedicarboxylate (19). To a solution of 655 mg (1.48 mmol) of bromide **15** in 35 mL of dry THF was added dropwise at room temperature 17 mL of 0.093 M sodiomethyl acetoacetate (1.6 mmol) in THF [prepared by addition of 0.35 mL of methyl acetoacetate (0.38 g, 3.2 mmol) to a suspension of 240 mg of 50% NaH dispersion in mineral oil]. After 2 h, the reaction was quenched by the addition of 20 mL of saturated NH_4Cl to the turbid mixture. The mixture was extracted with ethyl acetate, and the organic layer was washed with brine and dried (Na_2SO_4). Evaporation of the solvent in vacuo followed by chromatography of the residue on silica gel (55 g), eluting with ethyl acetate–hexane (2:3), produced 643 mg (91%) of β-keto ester **19** as a viscous yellow-green oil of sufficient purity for use in the next step: IR (film) 1740, 1600, 1530, 1360 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.28 (3 H, s), 3.42 (2 H, d, J = 6.99 Hz), 3.71 (3 H, s), 3.94 (3 H, s), 4.09 (3 H, s), 4.11 (3 H, s), 4.28 (3 H, s), 8.44 (1 H, s); mass spectrum, m/e (relative intensity) 478 (2.9), 463 (3.6), 432 (26.8), 400 (47.7), 390 (20.6), 43 (73.8), 28 (100).

Dimethyl 7,8-Dimethoxy-6-[3-methoxy-3-oxo-2-(phenylhydrazono)propyl]-5-nitro-2,4-quinolinedicarboxylate (20). To a stirred solution of 289 mg (0.604 mmol) of β-keto ester **19** in 20 mL of 50% aqueous pyridine at −10 to −5 °C was added 175 mg (0.912 mmol) of benzenediazonium fluoroborate over a period of 5 min. The reaction was quenched after 45 min by the addition of enough 12% HCl to neutralize the pyridine. The intermediate azo compound was extracted with ethyl acetate. The organic layer was washed with water and brine and dried (Na_2SO_4). Evaporation of the solvent in vacuo yielded 346 mg of a red oil. This material was dissolved in 10 mL of distilled methanol, and 26 mg (0.69 mmol) of sodium borohydride was added in portions over a period of 5 min. The mixture was stirred at room tem-

perature for 45 min, during which time a yellow precipitate was observed. The reaction was quenched by the addition of 10 mL of dilute HCl, and the solution was extracted with ethyl acetate. The organic layer was washed with water and brine and dried (Na_2SO_4). The solvent was removed in vacuo, and recrystallization of the crude product from ethyl acetate-hexane afforded 228 mg (70% yield) of **20** as a yellow solid: mp 169–170 °C; IR (KBr) 3320, 1720, 1705, 1600, 1570, 1530, 1350 cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.91 (3 H, s), 4.02 (3 H, s), 4.03 (3 H, s), 4.08 (3 H, s), 4.26 (5 H, s), 7.2 (5 H, m), 8.46 (1 H, s), 9.49 (1 H, s); mass spectrum, *m/e* (relative intensity) 540 (1.0), 494 (7.6), 462 (20.7), 435 (76.8), 105 (38.8), 92 (43.1), 77 (100); exact mass calcd for $C_{25}H_{24}N_4O_{10}$ – NO_2 523.1465, found 523.1451; calcd for $C_{25}H_{24}N_4O_{10}$ – NO_2 – CH_3O 463.1380, found 463.1376; calcd for $C_{25}H_{24}N_4O_{10}$ – PhN_2 435.1039, found 435.1028.

Trimethyl 4,5-Dimethoxy-1*H*-pyrrolo[2,3-*f*]quinoline-2,7,9-tricarboxylate (21). A solution of 38.9 mg (0.0720 mmol) of hydrazone **20** in 35 mL of distilled methanol containing 0.2 mL of 5% HCl and 60 mg of 5% Pd/C was stirred at room temperature under an atmosphere of H_2. The theoretical uptake of hydrogen was complete after 2 h, and the catalyst was removed by filtration. The solution was brought to neutrality with $NaHCO_3$ and was extracted with ethyl acetate. The organic layer was washed with brine and dried (Na_2SO_4). Evaporation of the solvent in vacuo yielded 19.0 mg (66%) of triester **21** of sufficient purity for use in the next step. A sample purified by preparative TLC, eluting with ethyl acetate/hexane (1:1), had the following: mp 220 °C; IR (KBr) 1710, 1600 cm^{-1}; ^1H NMR ($CDCl_3$) δ 4.01 (3 H, s), 4.09 (3 H, s), 4.13 (3 H, s), 4.17 (3 H, s), 4.33 (3 H, s), 7.51 (1 H, d, J = 2.4 Hz), 8.83 (1 H, s), 12.44 (1 H, br s); mass spectrum, *m/e* (relative intensity) 402 (23.7), 387 (90.1), 355 (100), 327 (33.7), 295 (27.9); exact mass calcd for $C_{19}H_{18}N_2O_8$ 402.1036, found 402.1064.

4,5-Dimethoxy-1*H*-pyrrolo[2,3-*f*]quinoline-2,7,9-tricarboxylic Acid (22). A solution of 32 mg (0.080 mmol) of triester **21** in 10 mL of methanol containing 1 mL of 5% aqueous KOH was refluxed for 15 h. The mixture was cooled, and a fine yellow solid precipitate formed. The solvent was removed in vacuo, and the solid residue was dissolved in a minimum amount of water and acidified with dilute HCl. An orange solid formed which was collected and purified by reverse-phase chromatography on a Waters Seppak C_{18} cartridge, yielding triacid **22**: IR (KBr) 3500–2500, 1710 cm^{-1}; ^1H NMR (D_2O, pH 7) δ 3.85 (3 H, s), 4.02 (3 H, s), 7.09 (1 H, s), 7.94 (1 H, s).

Trimethyl 4,5-Dihydro-4,5-dioxo-1*H*-pyrrolo[2,3-*f*]quinoline-2,7,9-tricarboxylate (23). To a suspension of 13 mg (0.032 mmol) of **21** in 1 mL of distilled THF were added 23.6 mg (0.191 mmol) of freshly prepared AgO and 10 drops of 6 N HNO_3.[23] After 10 min complete solution had occurred. The reaction was quenched by the addition of water, and the solution was extracted with CH_2Cl_2. The organic layer was washed with water and brine and dried (Na_2SO_4). The solvent was removed in vacuo, and the crude orange solid residue was purified by preparative TLC with chloroform–ethanol (95:5), yielding 8 mg (66%) of **23** as a bright orange solid: mp 220 °C dec; IR (KBr) 1725, 1718, 1685 cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.98 (3 H, s), 4.07 (3 H, s), 4.18 (3 H, s), 7.47 (1 H, s), 8.89 (1 H, s); mass spectrum, *m/e* (relative intensity) 374 (40.7), 372 (7.3), 344 (33.1), 342 (59.6), 314 (41.9), 286 (72.5), 282 (53), 254 (100); UV (MeOH) λ_{max} 372, 250 nm; exact mass calcd for $C_{17}H_{12}N_2O_8$ 372.0594, found 372.0576.

4,5-Dihydro-4,5-dioxo-1*H*-pyrrolo[2,3-*f*]quinoline-2,7,9-tricarboxylate (Methoxatin, 1). To a solution of 6.1 mg (0.016 mmol) of triester **23** in 15 mL of THF/water (85:15) was added 19.2 mg (0.802 mmol) of LiOH. After 5.5 h the reaction was quenched by the addition of dilute HCl until acidic, causing precipitation of a dark red solid. The solvent was removed in vacuo, and the solid residue was purified by reverse-phase chromatography on a Waters Seppak C_{18} cartridge with water followed by 30% aqueous methanol as the eluant, affording 4.1 mg (75%) of methoxatin (**1**) as a dark red solid: ^1H NMR (D_2O, pH 7) δ 7.15 (1 H, br s), 8.21 (1 H, vbr s); UV (H_2O, pH 7) λ_{max} 332, 268 (sh), 250 nm.

4,5-Dihydro-5-hydroxy-4-oxo-5-(2-oxopropyl)-1*H*-pyrrolo[2,3-*f*]quinoline-2,7,9-tricarboxylic Acid (2). A solution of 5 mg (0.015 mmol) of methoxatin (**1**) in 4.0 mL of acetone containing 1 mL of 1% aqueous NH_3 was stirred at room temperature for 0.5 h. The reaction was quenched by the addition of dilute HCl, and the mixture was filtered. The solvent was removed in vacuo, yielding 4.0 mg (70%) of aldol product **2** which was homogeneous by TLC and was identical with an authentic sample:[24] ^1H NMR (Me_2SO-d_6) δ 2.01 (3 H, s), 3.59 (1 H, d, J = 17.3 Hz), 4.00 (1 H, d, J = 17.3 Hz), 7.13 (1 H, d, J = 2.2 Hz), 8.41 (1 H, s), 13.40 (1 H, br); UV (H_2O) λ_{max} 360, 318, 252 nm.

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Synthesis of Nuclear Monobromobenz[*a*]anthracenes[1]

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The syntheses of 2-bromo-, 5-bromo-, 10-bromo-, and 11-bromo-7-methylbenz[*a*]anthracenes, of 5-bromo- and 7-bromo-12-methylbenz[*a*]anthracenes, and of 4-bromo- and 9-bromo-7,12-dimethylbenz[*a*]anthracenes are described. Bromination of 12-methylbenz[*a*]anthracene with tetramethylammonium chlorodibromide was superior to bromine in producing pure 7-bromo-12-methylbenz[*a*]anthracene.

The objective of the research to be described is to synthesize all of the nuclear monobromo derivatives of 7-methylbenz[*a*]anthracene (7-MBA, **1**), 12-methylbenz[*a*]anthracene (12-MBA, **2**), and 7,12-dimethylbenz[*a*]anthracene (DMBA, **3**). The hydrocarbons **1**–**3** represent a planar compound, **1**, of high carcinogenic activity,[3] a nonplanar[4] analogue, **2**, of low carcinogenic activity,[3] and a nonplanar[4] compound, **3**, of the highest carcinogenic

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