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SYNTHESIS OF THE BACTERIAL COENZYME METHOXATIN †

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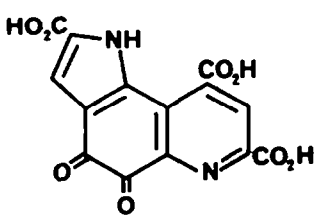
Summary. A short total synthesis of the bacterial coenzyme methoxatin (1) (4,5-dihydro-4,5-dioxo-1H-pyrrolo[2,3-f]quinoline-2,7,9-tricarboxylic acid) is described. The route involves the two step conversion of 4-acetamido-2-benzyloxybenzaldehyde (5b) into methyl 6-acetamido-4-benzyloxyindole-2-carboxylate (7b) (74%), followed by regioselective annulation of the third ring (55%), and debenzylation and oxidation with benzoyl t-butyl nitroxide to give the tricyclic quinone triester (13) (methoxatin triester) (83%).

Methylotrophic bacteria, which can utilise methane or methanol as their sole source of carbon, are of current importance as they are used in the production of single cell protein.[1] These bacteria contain a methanol dehydrogenase which possesses a unique cofactor, quite different from the normal redox coenzymes such as flavins and nicotinamide.[2] Although for a long time the coenzyme was thought to be a pteridine,[3] subsequent e.s.r. spectroscopic studies suggested that it was a quinone,[4] and the structure was finally elucidated as 4,5-dihydro-4,5-dioxo-1H-pyrrolo[2,3-f]quinoline-2,7,9-tricarboxylic acid (1), trivial names methoxatin or PQQ, by an X-ray crystallographic study of its aldol adduct (2, Nu = CH_2COCH_3) with acetone.[5] Other nucleophiles such as amines, alcohols, and urea also readily give the corresponding hemiketal adducts (2),[6] indicating the high reactivity of C-5 in methoxatin.

Once the structure of methoxatin was known, interest in its mechanism of action as a cofactor rapidly developed.[7] This interest has been fuelled by the commercial importance of methylotrophic bacteria,[1] by the use of methoxatin in the non-enzymic oxidation of amines and alcohols,[8] and by the discovery that methoxatin is more widely distributed in Nature than had been originally thought, occurring as the cofactor in a number of other dehydrogenases from a variety of



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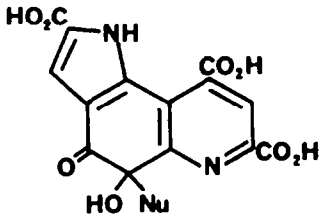

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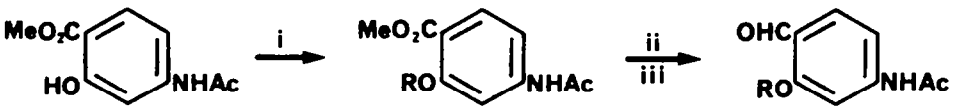
† DEDICATED WITH RESPECT AND AFFECTION TO PROFESSOR R.A. RAPHAEL ON THE OCCASION OF HIS SIXTY-FIFTH BIRTHDAY

organisms.[2] Recently the first example of a mammalian enzyme which contains methoxatin has been reported.[9] The enzyme, bovine serum amine oxidase, is similar to one found in humans, and is responsible for the conversion of spermine and spermidine into their corresponding aldehydes. The biosynthetic pathway to methoxatin remains unknown, and therefore it is not known whether the coenzyme can be synthesised by mammals, causing speculation as to its role as a hitherto unsuspected vitamin.[9]

The biological interest together with the unusual tricyclic ortho-quinonoid structure have made methoxatin an attractive target for total synthesis, particularly since only small quantities of the coenzyme are available from natural sources. At the outset of our work two syntheses had been reported, by Corey[10] and by Weinreb,[11] whilst a third from Hendrickson[12] appeared during the course of our studies.[13] Recently a fifth synthesis of the coenzyme, starting from derivatives of hypothetical biosynthetic precursors, has been published by Büchi.[14]

RESULTS AND DISCUSSION

The overall synthetic strategy involves, as a key step, formation of the indole ring from a readily available benzaldehyde, followed by annulation of the pyridine ring. This conversion of benzaldehydes into indoles proceeds in just two steps via the corresponding azidocinnamates,[15,16] and is far superior to the classical Reissert synthesis as a route to indole-2-carboxylates. The starting benzaldehydes (5) were prepared (Scheme 1) from the known[17] methyl 4-acetamido-2-hydroxybenzoate (3), obtained by esterification and N-acetylation of commercially available 4-aminosalicyclic acid, by O-alkylation followed by reduction of the ester to the corresponding benzyl alcohol with lithium aluminium hydride, and reoxidation to the benzaldehyde with manganese (IV)oxide or barium manganate. It is noteworthy that in these particular cases, the direct reduction of the ester to the aldehyde using diisobutylaluminium hydride was much less satisfactory than the alternative two step procedure. The overall yields of the benzaldehydes (5a) and (5b) from 4-aminosalicylic acid were 63 and 67% respectively.



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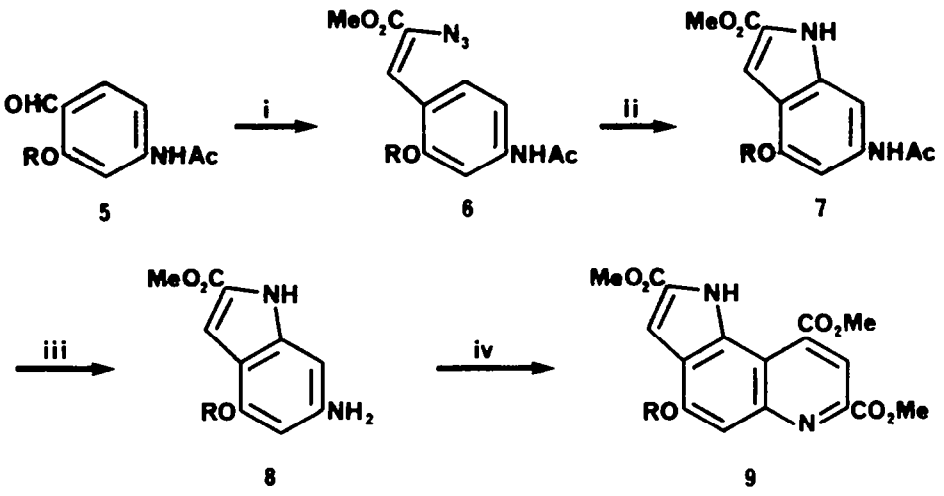
Scheme 1. (a, R = Me; b, R = CH_2Ph). Reagents: i, MeI or $PhCH_2Br$, K_2CO_3, acetone; ii, $LiAlH_4$, THF; iii, MnO_2 or $BaMnO_4$, $CHCl_3$.

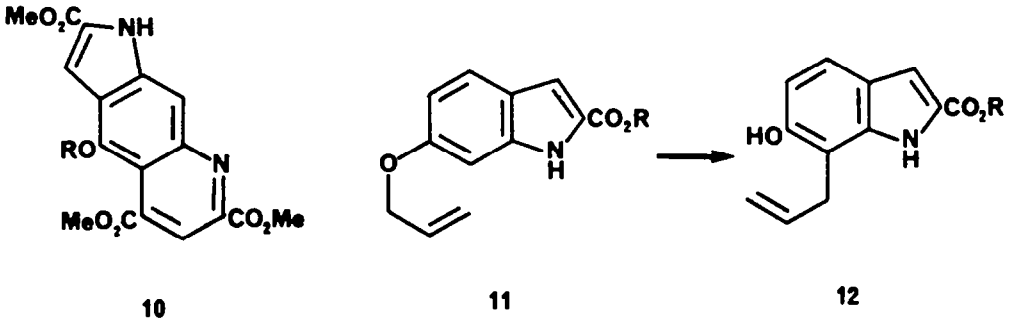
The aldehyde (5a) was condensed with methyl azidoacetate to give the azidocinnamate (6a), thermolysis of which in boiling xylene gave the expected indole (7a) [60% from the aldehyde (5a)]. In a similar manner, the benzaldehyde (5b) was converted into the indole (7b), although in slightly higher overall yield (74%). The indole (7a) was elaborated to the required tricyclic pyrroloquinoline (9a) in 62% overall yield by acid cleavage of the acetamide followed by reaction of the resulting aniline (8a) with commercially available dimethyl 2-oxoglutaconate in a Doebner - von Miller type quinoline synthesis, similar to that used by Corey and Tramontano in their synthesis of methoxatin.[10] In an identical two step sequence the tricyclic triester (9b) was prepared from the indole (7b) in 55% yield (Scheme 2).

Although the 6-aminoindole (8) has two possible sites (C-5 and C-7) for cyclisation, in neither experiment was there any trace of the alternative 'linear' pyrroloquinoline (10) formed by

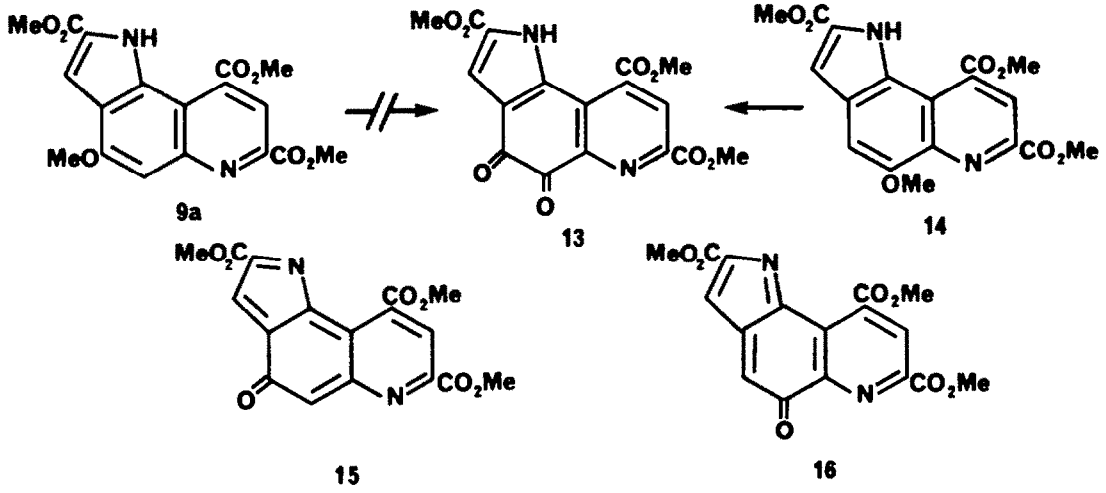


Scheme 2. (a, R = Me; b, R = CH_2Ph) Reagents: i, $MeO_2CCH_2N_3$, NaOMe, MeOH; ii, xylene, reflux; iii, MeOH, HCl, heat; iv, $MeO_2CCOCH{=}CHCO_2Me$, CH_2Cl_2, then H^+.

cyclisation to the indole 5-position. The fact that cyclisation would occur completely regioselectively to the 7-position to give the required 'angular' pyrroloquinoline (9) was expected on the basis of 'bond fixation' in indoles. This 'bond fixation' is also indicated by the regioselective Claisen rearrangement of 6-allyloxyindole-2-carboxylates (11) to 7-allyl-6-hydroxyindole-2-carboxylates (12).[18,19]

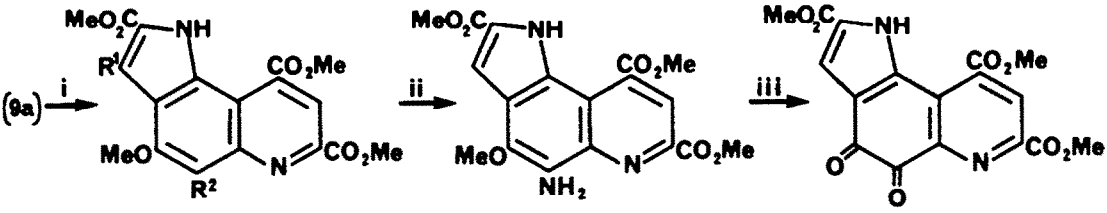


The key tricyclic intermediate (9) is obtained in just four steps from the benzaldehyde (5), and therefore it remained only to introduce the ortho-quinone unit by oxidation of the central ring. All attempts to oxidise the 4-methoxypyrroloquinoline (9a) directly to the quinone (13) were unsuccessful. This compound failed to react with cerium (IV) ammonium nitrate (CAN) even under forcing conditions, in direct contrast to the 5-methoxy isomer (14) which was oxidised to the quinone (13) using the same reagent under mild conditions.[10] This marked difference in reactivity is ascribed to the relative stabilities of the likely intermediates in the oxidation, the one (15) derived from the 4-methoxy isomer (9a) being considerably less stable than the isomeric intermediate (16) derived from the easily oxidised 5-methoxy isomer (14). Although CAN is probably the most commonly used reagent to effect oxidative demethylation of methoxyarenes, other reagents can be used. However, the 4-methoxypyrroloquinoline (9a) did not react with nitric acid,[20] nitrous acid,[20] silver (II) oxide,[21] or manganese (IV) oxide impregnated with nitric acid,[22] and therefore no further attempts at direct oxidation of (9a) were made.



9a 13 14 15 16

Although the methoxypyrroloquinoline (**9a**) could not be directly oxidised to the quinone (**13**), the desired transformation could be carried out indirectly (Scheme 3) via the 4-methoxy-5-nitro compound (**17**). This nitro compound was obtained in 41% yield after chromatographic separation from the 3-nitro isomer (**18**) and the 3,5-dinitro compound (**19**), by nitration of (**9a**) using ammonium nitrate in trifluoroacetic anhydride (TFAA). These nitrating conditions[23] gave the highest proportion of the desired 5-nitro isomer, other conditions such as sodium nitrate in concentrated sulphuric acid and standard 'mixed acid' giving high yields of the 3-nitro (**18**) and 3,5-dinitro (**19**) compounds respectively. Presumably under such conditions the pyrroloindole is completely protonated thereby reducing electron density at C-5 more than at C-3. In essentially neutral TFAA, however, electron release from the methoxy group causes the reactivity of C-5 and C-3 to be finely balanced. Conversion of the 5-nitro compound (**17**) into the ortho-quinone(**13**) was achieved in 68% yield by catalytic hydrogenation to the corresponding amine (**20**) followed by oxidation with manganese (IV) oxide in sulphuric acid at 0°C.[12]



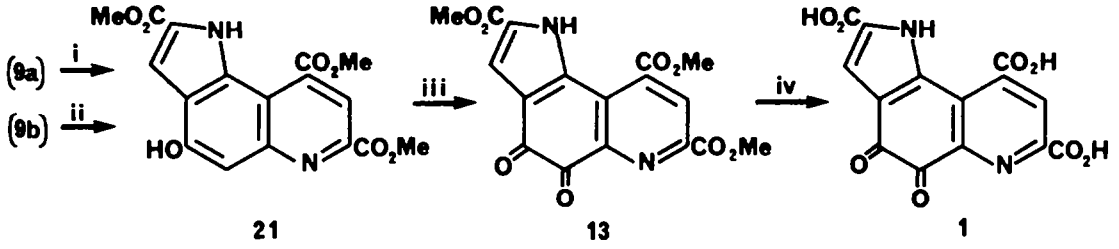
(**17**) R^1 = H, R^2 = NO_2
(**18**) R^1 = NO_2, R^2 = H
(**19**) R^1 = R^2 = NO_2

20 **13**

Scheme 3. Reagents: i, NH_4NO_3, TFAA; ii, H_2, Pd-C, MeOH; iii, MnO_2, 35% H_2SO_4, 0°C.

Since the overall yield (29%) of the quinone (**13**) from the 4-methoxypyrroloquinoline (**9a**) (Scheme 3) was disappointing, an alternative was sought. Although the 4-methoxy compound (**9a**) could not be oxidised directly to the quinone (**13**), it seemed likely that the corresponding phenol (**21**) would be oxidised more readily. After much experimentation it was found that the pyrroloquinoline (**9a**) could be demethylated by treatment with an excess of boron tribromide in dichloromethane at room temperature to give, after reesterification, the phenol (**21**) in poor yield (27%). In contrast, however, the 4-benzyloxypyrroloquinoline (**9b**) proved to be excellent precursor for the phenol (**21**), which was obtained in 89% yield by catalytic hydrogenolysis. Initial attempts to oxidise the phenol (**21**) using Fremy's salt, often the reagent of choice for the oxidation of

phenols to ortho- and para-quinones, were unsatisfactory because of the insolubility of the hydroxypyrroloquinoline in the aqueous phosphate buffer reaction medium. However, using the organic-soluble nitroxide, benzoyl t-butyl nitroxide,[24] in dichloromethane-methanol (9:1), the phenol (21) was efficiently oxidised to the required quinone (13) in 93% yield (Scheme 4). Thus the quinone triester (13) (methoxatin triester) is available in six steps from the benzaldehyde (5b) via the indole (7b) and the pyrroloquinoline (9b) in an overall yield of 34%.



21 13 1

Scheme 4. Reagents: i, BBr_3, CH_2Cl_2, then MeOH, H^+; ii, H_2, Pd-C, MeOH; iii $Bu^t(COPh)NO$, CH_2Cl_2-MeOH (9:1); iv, $HC(OMe)_3$, MeOH, H^+, then aq. K_2CO_3, 85°C, then HCl to pH 2.5.

Finally, a sample of the quinone triester (13) was converted into methoxatin (1) itself using a method described in the literature.[10] The resulting synthetic coenzyme was identical with authentic material obtained from natural sources.

EXPERIMENTAL

For general points see refs. 16 and 18.

Methyl 4-Acetamido-2-hydroxybenzoate (3).- Concentrated sulphuric acid (34 ml) was added to a solution of 4-aminosalicyclic acid (20 g, 0.131 mol) in methanol (550 ml), and the resulting solution heated under reflux for 20 h. The solution was concentrated to ca. 150 ml, water (400 ml) was added, and the resulting suspension was neutralised with solid sodium hydrogen carbonate. The product was collected by filtration, washed with water (300 ml), and dried in vacuo to give methyl 4-amino-2-hydroxybenzoate (19.2 g, 88%) as colourless needles, m.p. 120-121°C (lit.,[25] 123-125°C).

Acetic anhydride (13.3 g, 0.13 mol) was added dropwise to a stirred solution of the above ester (21.7 g, 0.13 mol) in ethanol (300 ml) at 45°C. The mixture was heated for a further 3.5 h at 45°C, cooled, and then poured into water (1000 ml). The product was collected by filtration and dried in vacuo to give the title compound (3) (25.63 g, 94%) as colourless needles, m.p. 150-153°C (lit.,[17] 152-153°C).

Methyl 4-Acetamido-2-methoxybenzoate (4a).- A stirred mixture of the hydroxy ester (3) (15 g, 0.072 mol), anhydrous potassium carbonate (49.6 g, 0.359 mol), and iodomethane (102 g, 0.718 mol) in acetone (300 ml) was heated under reflux for 8 h. After cooling, the mixture was filtered and the filtrate evaporated to give the title compound (4a) (15.5 g, 97%) as a colourless solid, m.p. 131-133°C (lit.,[17] 127°C).

4-Acetamido-2-methoxybenzaldehyde (5a).- A solution of the ester (4a) (3.45 g, 0.016 mol) in dry THF (20 ml) was added to a stirred suspension of lithium aluminium hydride (1.18 g, 0.031 mol) in THF (40 ml) at 0°C. The mixture was stirred at 0°C for 2 h, and then quenched by the careful addition of saturated sodium sulphate solution. The solution was decanted from inorganic material, dried ($MgSO_4$) and evaporated to give 4-acetamido-2-methoxybenzyl alcohol (3.0 g, 98%) as

a pale yellow solid, m.p. 137-139°C (Found: C, 61.5; H, 6.65; N, 7.1. $C_{10}H_{13}NO_3$ requires C, 61.5; H, 6.7; N, 7.2%); ν_{max} 3480, 3440, 3330, 1660, 1610, and 1600 cm^{-1}; δ (90 MHz; $CDCl_3$) 2.07 (3H, s), 3.75 (3H, s), 4.57 (2H, s), 6.98 (1H, dd, J 8, 2 Hz), 7.22 (1H, d, J 8 Hz), 7.40 (1H, d, J 2 Hz), and 8.99 (1H, br s); OH not observed; m/z 195 (M^+) and 153 (base).

Activated manganese (IV) oxide (46 g, 0.56 mol) was added to a solution of the above benzyl alcohol (7.17 g, 0.037 mol) in chloroform (200 ml) and methanol (5 ml). The resulting suspension was stirred at room temperature for 20 h, filtered through Celite, and the filtrate evaporated to give a pale brown oil. This material was purified by chromatography (EtOAc eluant) to give the title compound (**5a**) (5.64 g, 80%) as a pale yellow solid, m.p. 149-151°C (lit.,[26] 141-142°C) (Found: C, 62.3; H, 5.75; N, 7.2. Calc. for $C_{10}H_{11}NO_3$: C, 62.2; H, 5.7; N, 7.25%).

Methyl 4-Acetamido-2-benzyloxybenzoate (**4b**).- A stirred mixture of the hydroxy ester (**3**) (20.75 g, 0.1 mol), anhydrous potassium carbonate (68.6 g, 0.496 mol), and benzyl bromide (21 ml, 0.177 mol) in acetone (400 ml) was heated under reflux for 24 h. After cooling, the mixture was filtered and the filtrate evaporated to an oil. Trituration with light petroleum gave the title compound (**4b**) (27.34 g, 92%) as colourless prisms, m.p. 113-115°C (Found: C, 68.3; H, 5.8; N, 4.6. $C_{17}H_{17}NO_4$ requires C, 68.2; H, 5.7; N, 4.7%); ν_{max} 3360, 1710, 1680, and 1620 cm^{-1}; δ (90 MHz; $CDCl_3$) 2.14 (3H, s), 3.90 (3H, s), 5.10 (2H, s), 6.95 (1H, dd, J 8, 2 Hz), 7.20-7.50 (5H, m), 7.61 (1H, d, J 2 Hz), 7.78 (1H, d, J 8 Hz), and 8.15 (1H, br s); m/z 299 (M^+), 267, and 91 (base).

4-Acetamido-2-benzyloxybenzaldehyde (**5b**).- The ester (**4b**) (25 g, 0.084 mol) was reduced with lithium aluminium hydride (6.35 g, 0.168 mol) as described for (**4a**) to give 4-acetamido-2-benzyloxybenzyl alcohol (18.68 g, 83%) as colourless rods, m.p. 115-116°C (Found: C, 70.7; H, 6.3; N, 5.2. $C_{16}H_{17}NO_3$ requires C, 70.85; H, 6.3; N, 5.2%); ν_{max} 3280, 1655, and 1610 cm^{-1}; δ [90 MHz; $(CD_3)_2CO$] 2.15 (3H, s), 3.35 (1H, t, J 6 Hz, D_2O exch), 4.70 (2H, d, J 6 Hz), 5.10 (2H, s), 6.97 (1H, dd, J 8, 2 Hz), 7.20-7.60 (7H, m), and 8.82 (1H, br); m/z 271 (M^+) and 91 (base).

Barium manganate (50 g, 0.195 mol) was added to a stirred solution of the above benzyl alcohol (5 g, 0.019 mol) in chloroform (250 ml), and the resulting suspension heated under reflux for 1 h. After cooling, the mixture was filtered through Celite, and the filtrate evaporated to give the title compound (**5b**) (5.0 g, 100%) as a colourless solid, m.p. 156-158°C, (Found: C, 71.1; H, 5.7; N, 5.25. $C_{16}H_{15}NO_3$ requires C, 71.4; H, 5.6; N, 5.2%); ν_{max} 3270, 1700, 1670, and 1585 cm^{-1}; δ (250 MHz; $CDCl_3$) 2.21 (3H, s), 5.18 (2H, s), 6.77 (1H, dd, J 8, 2 Hz), 7.30-7.48 (5H, m), 7.73 (1H, br), 7.79 (1H, d, J 8 Hz), 7.90 (1H, d, J 2 Hz), and 10.42 (1H, s); m/z 269 (M^+) and 91 (base).

Methyl 3-(4-Acetamido-2-methoxyphenyl)-2-azidopropenoate (**6a**).- A solution of 4-acetamido-2-methoxybenzaldehyde (**5a**) (1.5 g, 7.8 mmol) and methyl azidoacetate (3.58 g, 31 mmol) in methanol (15 ml) was added dropwise over 45 min to a stirred solution of sodium methoxide [from sodium (0.715 g, 31 mg atom)] in methanol (25 ml) at -15°C. After a further 2 h at -15°C, the mixture was warmed to room temperature, poured into saturated ammonium chloride solution (100 ml), and extracted with ethyl acetate (4 x 100 ml). The combined organic extracts were washed with water (3 x 150 ml), dried ($MgSO_4$), and evaporated to give a brown solid. Trituration with ice-cold methanol gave the title compound (**6a**) (1.65 g, 73%) as a yellow solid, m.p. 135-138°C (decomp.) (Found: C, 53.5; H, 4.8; N, 19.0. $C_{13}H_{14}N_4O_4$ requires C, 53.8; H, 4.8; N, 19.3%); ν_{max} 3320, 3260, 3200, 2130, 1720, 1675, and 1600 cm^{-1}; δ [250 MHz; $(CD_3)_2SO$] 2.03 (3H, s), 3.78 (3H, s), 3.82 (3H, s), 7.14 (1H, d, J 9 Hz), 7.21 (1H, s), 7.47 (1H, s), 8.13 (1H, d, J 9 Hz), and 10.18 (1H, br); m/z 262 (M^+ -28), 193 (base), and 150.

Methyl 6-Acetamido-4-methoxyindole-2-carboxylate (**7a**).- A suspension of the azide (**6a**) (1.26 g) in xylene (300 ml) was heated rapidly to reflux. After 4 h at reflux, the solution was cooled to room temperature whereupon the product crystallised. Filtration gave the title compound (**7a**)

(0.994 g, 87%) as pale yellow prisms, m.p. 241-242°C (Found: C, 59.4; H, 5.4; N, 10.6. $C_{13}H_{14}N_2O_4$ requires C, 59.5; H, 5.3; N, 10.7%); ν_{max} 3420-3100, 1700, 1670, 1630, and 1600 cm^{-1}; δ [90 MHz; $(CD_3)_2SO$] 2.08 (3H, s), 3.85 (3H, s), 3.88 (3H, s), 6.70 (1H, br s), 7.03 (1H, d, J 3 Hz), 7.62 (1H, br s), 9.89 (1H, br), and 11.72 (1H, br); m/z 262 (M^+), 224, 193, 151, and 150 (base).

Methyl 6-Amino-4-methoxyindole-2-carboxylate (8a).- A solution of the 6-acetamidoindole (7a) (0.994 g) in methanol (300 ml) presaturated with hydrogen chloride was heated under reflux for 6 h. After cooling, the solution was concentrated to ca. 75 ml, and then poured into a mixture of saturated sodium hydrogen carbonate (100 ml) and ethyl acetate (100 ml). The organic layer was separated, dried ($MgSO_4$), and evaporated to give the title compound (8a) (0.706 g, 85%) as a yellow solid which rapidly darkened in air, δ [90 MHz; $(CD_3)_2CO$] 3.84 (3H, s), 3.87 (3H, s), 6.08 (1H, d, J 2 Hz), 6.35 (1H, d, J 2 Hz), 7.15 (1H, d, J 3 Hz), and 10.90 (1H, br), NH_2 not observed.

Trimethyl 4-Methoxy-1H-pyrrolo[2,3-f]quinoline-2,7,9-tricarboxylate (9a).- Dimethyl 2-oxoglutaconate (352 mg, 2.05 mmol) was added to a solution of the 6-aminoindole (8a) (300 mg, 1.36 mmol) in dichloromethane (60 ml), and the resulting mixture stirred at room temperature for 12 h. One drop of a saturated solution of hydrogen chloride in ether was added, and the mixture stirred for a further 12 h. The mixture was diluted with chloroform (20 ml), washed with saturated sodium hydrogen carbonate solution (50 ml), dried ($MgSO_4$), and evaporated to a brown solid. Trituration with hot methanol gave the title compound (9a) (371 mg, 73%) as bright yellow microcrystals, m.p. 270-274°C (decomp.), (Found: C, 58.0; H, 4.3; N, 7.4. $C_{18}H_{16}N_2O_7$ requires C, 58.1; H, 4.3; N, 7.5%); ν_{max} 3300, 1720, and 1605 cm^{-1}; δ (250 MHz; $CDCl_3$) 4.01 (3H, s), 4.10 (3H, s), 4.12 (3H, s), 4.18 (3H, s), 7.32 (1H, s), 7.50 (1H, d, J 1 Hz), 8.78 (1H, s), and 12.58 (1H, br); m/z 372 (M^+).

Methyl 3-(4-Acetamido-2-benzyloxyphenyl)-2-azidopropenoate (6b).- A solution of 4-acetamido-2-benzyloxybenzaldehyde (5b) (5.0 g, 19 mmol) and methyl azidoacetate (13.8 g, 120 mmol) in THF (50 ml) was added dropwise to a cooled solution of sodium methoxide [from sodium (2.75 g, 120 mg atom)] in methanol (80 ml) maintaining the temperature in the range -10°C to -5°C. The mixture was stirred for a further 2 h at -10°C and then for 16 h at 2°C. Work-up as described for compound (6a) gave the title compound (6b) (6.13 g, 90%) as a pale yellow solid, m.p. 101-104°C (decomp.) (Found: C, 62.2; H, 4.85; N, 15.05. $C_{19}H_{18}N_4O_4$ requires C, 62.3; H, 4.9; N, 15.3%); ν_{max} 3320, 2120, 1700, 1665, 1605, and 1585 cm^{-1}; δ (90 MHz; $CDCl_3$) 2.10 (3H, s), 3.82 (3H, s), 5.04 (2H, s), 6.75 (1H, dd, J 8, 2.5 Hz), 7.16-7.68 (8H, m), and 8.09 (1H, d, J 8 Hz); m/z 338 (M^+ -28) and 91 (base).

Methyl 6-Acetamido-4-benzyloxyindole-2-carboxylate (7b).- Thermolysis of the azide (6b) (3.00 g) in xylene (350 ml) under the conditions described for azide (6a) gave the title compound (7b) (2.26 g, 82%) as a tan solid, m.p. 263-265°C (Found: C, 67.5; H, 5.4; N, 8.3. $C_{19}H_{18}N_2O_4$ requires C, 67.5; H, 5.3; N, 8.3%); ν_{max} 3320, 1720, and 1600 cm^{-1}; δ [250 MHz; $(CD_3)_2SO$] 2.04 (3H, s), 3.82 (3H, s), 5.19 (2H, s), 6.73-7.72 (8H, m), 9.98 (1H, br), and 11.85 (1H, br); m/z 338 (M^+) and 91 (base).

Methyl 6-Amino-4-benzyloxyindole-2-carboxylate (8b).- Acid methanolysis of the 6-acetamidoindole (7b) (1.85 g) under the conditions described for compound (7a) gave, after chromatography, the title compound (8b) (0.944 g, 58%) as a tan solid, m.p. 217-220°C (decomp.) (Found: C, 69.05; H, 5.5; N, 9.55. $C_{17}H_{16}N_2O_3$ requires C, 68.9; H, 5.4; N, 9.5%); ν_{max} 3430, 3370, 1680, 1630, and 1580 cm^{-1}; δ (250 MHz; $CDCl_3$) 3.88 (3H, s), 5.15 (2H, s), 6.04 (1H, br s), 6.26 (1H, br s), 7.27-7.57 (6H, m), and 8.52 (1H, br s), NH_2 not observed; m/z 296 (M^+) and 91 (base).

Trimethyl 4-Benzyloxy-1H-pyrrolo[2,3-f]quinoline-2,7,9-tricarboxylate (9b).- Reaction of the 6-aminoindole (8b) with dimethyl 2-oxoglutaconate under the conditions described for the indole (8a) gave the title compound (95%) as a bright yellow solid, m.p. 215-217°C (Found: C, 64.65; H,

4.6; N, 6.3. $C_{24}H_{20}N_2O_7$ requires C, 64.3; H, 4.5; N, 6.25%); ν_{max} ($CHCl_3$) 3310, 1720, and 1600 cm^{-1}; δ (250 MHz; $CDCl_3$) 4.00 (3H, s), 4.09 (3H, s), 4.14 (3H, s), 5.35 (2H, s), 7.36-7.56 (7H, m), 8.76 (1H, s), and 12.29 (1H, br s); m/z 448 (M^+, base).

Nitration of the Pyrroloquinoline (9a). (a) With sodium nitrate in sulphuric acid.- Sodium nitrate (12 mg, 0.14 mmol) was added to a solution of the pyrroloquinoline (9a) (50 mg, 0.13 mmol) in sulphuric acid (96%; 2 ml) at 0°C. After 1.5 h at 0°C, the mixture was poured into ice (10 g), neutralised with sodium hydrogen carbonate, and extracted with chloroform (3 x 10 ml). The combined extracts were dried ($MgSO_4$) and evaporated to give trimethyl 4-methoxy-3-nitro-1H-pyrrolo[2,3-f]quinoline-2,7,9-tricarboxylate (18) (56 mg, 100%) as yellow crystals, m.p. 277-280°C (Found: C, 51.7; H, 3.6; N, 10.1. $C_{18}H_{15}N_3O_9$ requires C, 51.8; H, 3.6; N, 10.1%); ν_{max} 3200, 1740, 1720, 1705, 1610, 1580, 1535, and 1360 cm^{-1}; δ (250 MHz; $CDCl_3$) 4.02 (3H, s), 4.06 (3H, s), 4.13 (3H, s), 4.19 (3H, s), 7.41 (1H, s), 8.87 (1H, s), and 13.17 (1H, br); m/z 417 (M^+) 277 (base), and 201.

(b) With mixed acid.- Fuming nitric acid (1 drop) was added to a solution of the pyrroloquinoline (9a) (20 mg) in sulphuric acid (96%; 2 ml) and the mixture stirred at 0°C for 0.5 h. Aqueous work up as above gave trimethyl 3,5-dinitro-4-methoxy-1H-pyrrolo[2,3-f]quinoline- 2,7,9-tricarboxylate (19) (24.1 mg, 97%), m.p. 253-254°C (Found: M^+ 462.0657. $C_{18}H_{14}N_4O_{11}$ requires 462.0659); ν_{max} 3210, 1750, 1710, 1610, and 1540 cm^{-1}; δ (250 MHz; $CDCl_3$) 4.05 (3H, s), 4.08 (3H, s), 4.19 (3H, s), 4.22 (3H, s), 9.01 (1H, s), and 13.37 (1H, br); m/z 462 (M^+), 417, 372 (base), 359, 314, and 282.

(c) With ammonium nitrate in TFAA.- A suspension of the pyrroloquinoline (9a) (20 mg, 0.054 mmol) in TFAA (3 ml) was stirred at room temperature for 2 h. Ammonium nitrate (4.3 mg, 0.054 mmol) was added, and the mixture stirred for a further 3.75 h. Aqueous work-up as above gave a yellow solid (29.1 mg), chromatography of which gave (i) starting material (9a) (ca. 1.3 mg, ca. 6% recovery), (ii) the 3-nitro compound (18) (5.8 mg), (iii) the 3,5-dinitro compound (19) (5.2 mg) and (iv) trimethyl 4-methoxy-5-nitro-1H-pyrrolo[2,3-f]quinoline-2,7,9-tricarboxylate (17) (8.7 mg, 41% based on 94% conversion), m.p. 263-265°C (Found: M^+ 417.0824. $C_{18}H_{15}N_3O_9$requires 417.0808); ν_{max} 3280, 1720, 1610, 1535, and 1360 cm^{-1}; δ (250 MHz; $CDCl_3$) 4.05 (6H, s), 4.20 (3H, s), 4.36 (3H, s), 7.67 (1H, d, J 2.8 Hz), 8.90 (1H, s), and 12.99 (1H, br); m/z 417 (M^+, base), 387, 359, and 327.

Trimethyl 5-Amino-4-methoxy-1H-pyrrolo[2,3-f]quinoline-2,7,9-tricarboxylate (20).- A suspension of the nitro compound (17) (60 mg) and 10% palladium-on-charcoal (30 mg) in methanol (120 ml) was shaken under an atmosphere of hydrogen for 20 h. The mixture was filtered through Celite, the filtrate evaporated, and the residue chromatographed to give the title compound (20) (52 mg, 93%) as a purple solid, m.p. 202-204°C (Found: C, 55.9; H, 4.5; N, 10.9. $C_{18}H_{17}N_3O_7$ requires C, 55.8; H, 4.4; N, 10.85%); ν_{max} 3340, 1720, and 1625 cm^{-1}; δ (250 MHz; $CDCl_3$) 4.00 (3H, s), 4.07 (3H, s), 4.09 (3H, s), 4.16 (3H, s), 4.92 (2H, br), 7.36 (1H, d, J 2 Hz), 8.82 (1H, s), and 12.24 (1H, br); m/z 387 (M^+), 372, 355, 340 (base), and 280.

Oxidation of the Amino Pyrroloquinoline (20).- Manganese (IV) oxide (44 mg) was added to a solution of the pyrroloquinoline (20) (52 mg) in sulphuric acid (35%; 10 ml) at 0°C. The mixture was stirred at 0°C for 45 min, filtered through Celite, and the filtrate neutralised with sodium hydrogen carbonate and extracted with chloroform (3 x 10 ml). The combined extracts were dried ($MgSO_4$) and evaporated to give trimethyl 4,5-dihydro-4,5-dioxo-1H-pyrrolo[2,3-f]quinoline-2,7,9-tricarboxylate (13) (36.5 mg, 73%) as a bright orange solid, m.p. 265-269°C (decomp.) (lit.,[10] 260-263°C decomp., lit.,[11] 220°C decomp.) (Found: 54.6; H, 3.2; N, 7.45. Calc. for $C_{17}H_{12}N_2O_8$: C, 54.8; H, 3.2; N, 7.5%); ν_{max} 1720 and 1680 cm^{-1}; λ_{max} (MeOH) 251, 306, and 376 nm; δ (250 MHz; $CDCl_3$) 3.99 (3H, s), 4.08 (3H, s), 4.19 (3H, s), 7.48 (1H, d, J 1 Hz), 8.90 (1H, s), and 12.98 (1H, br); m/z 372 (M^+), 341, 314, 286, 282, and 254 (base).

Trimethyl 4-Hydroxy-1H-pyrrolo[2,3-f]quinoline-2,7,9-tricarboxylate (21). (a) By demethylation of (9a).- Boron tribromide (1M in CH_2Cl_2; 2 ml, 2 mmol) was added to the pyrroloquinoline (9a) (50 mg, 0.134 mmol) and the resulting red solution stirred at room temperature for 140 h. Methanol (20 ml) was added carefully, followed by sulphuric acid (96%; 1 ml), and the mixture heated under reflux for 48 h. After cooling, the mixture was poured onto saturated aqueous sodium hydrogen carbonate solution (30 ml), and extracted with chloroform (3 x 30 ml). The combined extracts were dried ($MgSO_4$), evaporated, and the residue chromatographed to give (i) the starting material (9a) (12 mg), and (ii) the title compound (21) (9.9 mg, 27% based on consumed starting material) as yellow microcrystals, m.p. 279-281°C (Found: C, 56.8; H, 4.1; N, 7.5. $C_{17}H_{14}N_2O_7$ requires C, 57.0; H, 3.9; N, 7.8%); ν_{max} 3290, 1700, and 1590 cm^{-1}; δ [250 MHz; $(CD_3)_2SO$] 4.00 (3H, s), 4.07 (3H, s), 4.18 (3H, s), 7.32 (1H, s), 7.54 (1H, d, J 2 Hz), 8.67 (1H, s), and 12.48 (1H, s), OH not observed; m/z 358 (M^+, base).

(b) By debenzylation of (9b).- A suspension of the pyrroloquinoline (9b) (0.677 g) and 10% palladium-on-charcoal (0.25 g) in methanol (250 ml) was shaken under an atmosphere of hydrogen for 20 h. The mixture was filtered through Celite, and the filtrate evaporated to give the title compound (21) (0.484 g, 89%) as a bright yellow solid identical with the material prepared above.

Oxidation of Hydroxy Pyrroloquinoline (21).- Benzoyl t-butyl nitroxide[24] (536 mg, 2.79 mmol) was added to a solution of the pyrroloquinoline (21) (100 mg, 0.279 mmol) in a mixture of methanol (30 ml) and chloroform (270 ml). The resulting dark green solution was stirred at room temperature for 16 h, evaporated, and the residue triturated with ether to give the quinone triester (13) (96.4 mg, 93%) as a bright orange solid, identical with material prepared previously.

Methoxatin (1) (4,5-Dihydro-4,5-dioxo-1H-pyrrolo[2,3-f]quinoline-2,7,9-tricarboxylic acid) [10].- A solution of the quinone triester (13) (10 mg) and trimethyl orthoformate (29 µl) in dry methanol (25 ml) containing a trace of toluene-4-sulphonic acid was heated under reflux for 3 h. The solvent was evaporated to leave a yellow solid. Potassium carbonate (0.5 M; 15 ml) was added, and the suspension heated at 85°C for 4 h. After cooling, the yellow solution was acidified to pH 2.5 with hydrochloric acid (6M). The resulting red precipitate was collected by centrifugation and dried in vacuo to give methoxatin (1) (7 mg, 79%), identical with material obtained from natural sources.

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