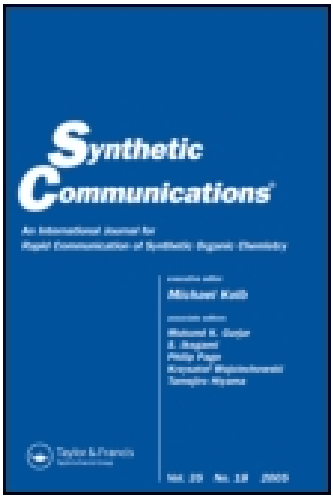

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Sean E. Brynjelsen [a] , Mark Doty [a] & Mitchell J. Poss [a]

[a] Baxter Healthcare , Route 120 and Wilson Rd, Round Lake, IL

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FACILE SYNTHESIS OF HEMATINIC ACID

Sean E. Brynjelsen, Mark Doty and Mitchell J. Poss*

Baxter Healthcare, Route 120 and Wilson Rd,
Round Lake, IL

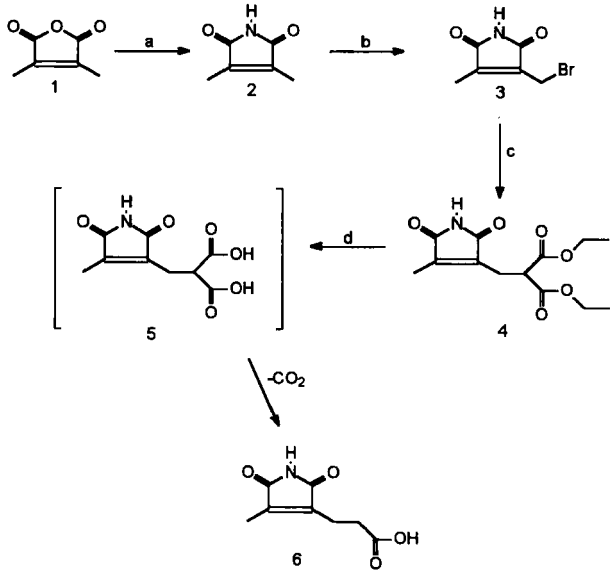
Abstract: *Hematinic acid is conveniently synthesized in four steps from commercially available 2,3-dimethylmaleic anhydride.*

Both the *in vitro* and *in vivo* oxidation of iron porphyrins in cytochrome P-450, hemoglobin, and other heme-containing proteins results in a variety of nitrogen containing degradation products[1-3]. Many of these have been identified as pyrroledione derivatives such as hematinic acid (6). The isolation of useful quantities of these compounds from the vertebrate systems in which they have been found is complicated by the facts that they are present in low concentration and must be isolated from complex matrices. Similar shortcomings apply to the products of studies of the oxidative degradation of hemoglobin or protoporphyrin heme[1-4]. Generally, this degradative approach has resulted in very low yields. Herein we report a four step synthesis for the generation of larger quantities of hematinic acid, one of the primary products of heme degradation. The synthetic strategy is outlined in Scheme I was based primarily on known chemistry. Each step resulted in high yields using commercially available reagents. The key step is the decarboxylation of the pyrroledione malonic acid (5) under mildly acidic conditions.

In the initial step dimethylmaleic anhydride is allowed to react with ammonium acetate in acetic acid to give the corresponding pyrroledione. Subsequent bromination using one

*author to whom correspondence should be addressed

SCHEME 1



Reagents: a. ammonium acetate, acetic acid, reflux; b. NBS, benzoyl peroxide, CCl_4, reflux ; c. diethyl malonate, sodium ethoxide, EtOH, reflux ; d. 1N hydrochloric acid, reflux.

equivalent of N-bromosuccinimide gave the monobrominated compound (3) in good yield (83%). Reaction with diethylmalonate converted (3) to the malonate derivative, which was then decarboxylated to give hematinic acid (6).

As shown in Scheme I, the conversion of the 3-(bromomethyl)-4-methyl-1H-pyrrole-2,5-dione to (4) required the use of diethylmalonate with an equimolar ratio of sodium ethoxide. Subsequent reflux in 1N HCl for 4 hours resulted in conversion of (4) directly to hematinic acid (6). The intermediate (5) was not detected upon workup of the reaction.

EXPERIMENTAL

Melting points were measured on a Fisher-Johns apparatus and were uncorrected. All GC/MS data were obtained on an HP5890/5971 system. NMR spectra (^1H @ 270.1 MHz and ^{13}C

@ 67.9MHz) were recorded on a Bruker 275 MHz instrument. Chemical shifts were defined relative to internal acetone. The preparative HPLC system was based on a C_{18} (22.5 x 300 mm, 27 mL/min) column using an acetonitrile/water (0.1% TFA) mobile phase with UV-VIS detection (230 nm). Purity determination of the final product was performed by differential scanning calorimetry (DSC) at a heating rate of 1°C/min.

Synthesis of 3,4-dimethyl-1H-pyrrole-2,5-dione [5] 2

A solution containing 2,3-dimethyl maleic anhydride (10.12 g, 80.3 mmol) in acetic acid (200 mL) was stirred continuously while adding ammonium acetate (8.2 g, 106 mmol) in 100 mL of acetic acid over a 5 minute period. The reaction was refluxed for 4 hours and then allowed to cool to room temperature. The solvent was removed to obtain a clear oil which subsequently crystallised. Recrystallization from hexane-benzene afforded the pure product (85%).

m.p. 111-112 °C, [lit[5]. m.p. 111-113 °C]; MS(EI): m/z 125, 54

Synthesis of 3-(bromomethyl)-4-methyl-1H-pyrrole-2,5-dione [6] 3

In a 200 mL round bottom flask **2** (5.2 g, 41.6 mmol) was combined with carbon tetrachloride (80 mL) to which N-bromosuccinimide (7.5 g, 42.1 mmol) and benzoyl peroxide (0.12 g) were added. The mixture was then refluxed for 4 hours with constant stirring. Once the reaction had cooled, the contents were analyzed by GC/MS which showed the major product to be the monobrominated compound **3**. The material was then purified by flash column chromatography by first eluting with chloroform (discard) and then with ethyl acetate (200 mL). The ethyl acetate fraction was concentrated to a yellow viscous oil. The product was further purified via preparative HPLC (65% water w/0.1%TFA and 35% acetonitrile) to a transparent viscous liquid (10.15 grams, 83%).

MS(EI): m/z 203, 124, 81, 53. ^{1}H-NMR (275 MHz, acetone-d_6): 2.06 (-CH_3), 4.38 (-CH_2Br) , 9.75 (-NH). ^{13}C NMR (275MHz, acetone-d_6): 8.9, 18.5, 137.4, 142.6, 171.0, 172.3 ppm.

Synthesis of diethyl-2-[(4-methyl-2,5-dioxo-2,5-dihydro-1H-3-pyrrolyl)methyl]malonate 4

A solution containing 21% sodium ethoxide (6.351 g, 19.6 mmol) and 50 mL ethanol was stirred for 1 minute. Diethyl malonate (3.140 g, 19.6 mmol) was added followed by a solution

containing **3** (4.0 g) in ethanol (50 mL). The mixture was refluxed for 2 hours and then allowed to cool to room temperature. The product was extracted with ethyl acetate (100 mL) and filtered through silica gel. Further purification by HPLC (60% water w/0.1% TFA and 15% acetonitrile) and concentration of the fractions of interest gave the product as a clear viscous oil (76%).

MS(EI): m/z 283(M^+), 238, 237, 192, 191, 164. NMR (275 MHz, acetone-d_6): 1.21 (t, 6H), 1.94 (s, 3H), 2.90 (d, 2H), 3.81 (t, 1H), 4.14 (q, 4H), 9.58 (s, 1H). ^{13}C NMR (275 MHz, acetone-d_6): 8.7, 14.4, 23.8, 50.6, 62.2, 138.0, 141.1, 169.0, 172.8 ppm.

Synthesis of 3-(4-methyl-2,5-dioxo-2,5-dihydro-1H-3-pyrrolyl)propanoic acid (common name hematinic acid) 6

In a round bottom flask 1N HCl (200 mL) was added to **4** (4.1 g, 14.5 mmol), and the solution refluxed with constant stirring for 4 hours. The reaction was allowed to cool and the solvent removed (without neutralization) to a yellow oil. Isolation by preparative HPLC (85% water w/~0.1%TFA and 15% acetonitrile) followed by solvent removal gave the pure product as a white solid (43%).

m.p. 113-115 °C, [lit[4]. m.p. 114-115 °C]. MS(EI) *TMS derivatized*: m/z 327 (M^+), 312, 237, 194. ^1H-NMR (275MHz, acetone-d_6): 1.99 (s, 3H), 2.65 (m, 4H), 9.47 (s, NH). ^{13}C NMR (275MHz, acetone-d_6): 8.6, 19.9, 32.1, 139.7, 140.6, 173.1, 173.2, 173.6 ppm. Anal. Calc. for $C_8H_9NO_4$: C 52.46 H 4.95 N 7.65. Found: C 52.16 H 4.87 N 7.54 DSC purity (mole%): 98.2% DSC m.p. 114.5°C

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