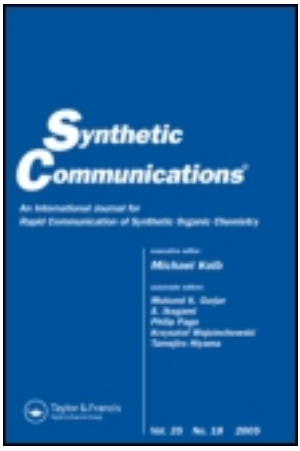

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Preparation of Naturally Occurring Anthraquinones

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PREPARATION OF NATURALLY OCCURRING ANTHRAQUINONES

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ABSTRACT

Several anthraquinones, which occur in plants used to treat cancer in traditional Chinese Medicine, have been synthesised and tested for cytotoxicity in two mammalian cell lines.

INTRODUCTION

Hedyotis diffusa (Rubiaceae) is a herb which has been used extensively in Traditional Chinese Medicine. This annual, which is grown in South East China, is used as a folk medicine for the treatment of a variety of diseases including appendicitis, hepatitis, tonsillitis, sore throat and urethral infection. It is also used as an antitumour "King" herb for the treatment of a number of human cancers such as hepatoma, cervical, gastric, and intestinal carcinoma[1-3].

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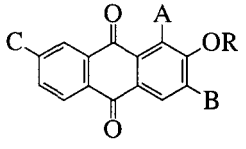
Hedyotis diffusa is known to contain iridoid glucosides[4-6] and steroids[3,7]. Also, four anthraquinones - 2-hydroxy-3-methyl (**1**), 2-methoxy-3-methyl (**2**), 2-hydroxy-1-methoxy-3-methyl (**3**) and 2,3-dimethoxy-6-methyl (**4**) 9,10-anthraquinone (Figure) - have been isolated[8] from *H. diffusa.* These four anthraquinones (**1-4**) have supposedly[8] shown antitumour effects: however no evidence of their anticancer properties has been published.



1 R = A = C = H; B = Me
2 R = B = Me; A = C = H
3 R = C = H; A = OMe; B = Me
4 R = C = Me; A = H; B = OMe

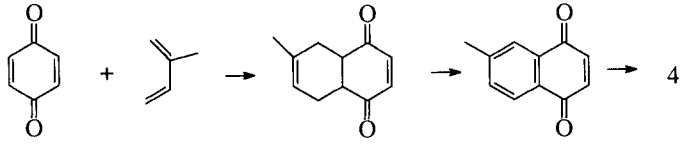
In general, apart from the clinically used anthracyclines, few[9] anthraquinones exhibit antitumour activity. In order to assess the cytotoxicity of the anthraquinones (**1** - **4**) syntheses of these four compounds were undertaken.

RESULTS AND DISCUSSION

A synthesis of the trisubstituted anthraquinone (**3**) ("digitolutein") has been described[10] previously, but lacked experimental detail. A synthesis of the dimethoxy anthraquinone (**4**) has also been described. This latter synthesis

involved the Diels-Alder reaction of benzoquinone (**5**) with isoprene (**6**) to yield the tetrahydronaphthoquinone (**7**). Oxidation of **7** afforded 6-methyl-1,4-naphthoquinone (**8**). Diels-Alder reaction of naphthoquinone (**8**) with 2,3-dimethoxy-1,3-butadiene the yielded anthraquinone (**4**) (Scheme 1).

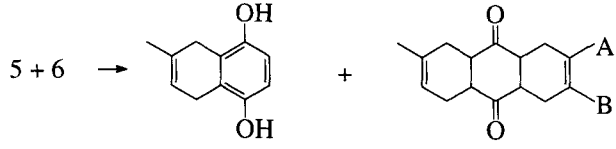
Scheme 1



5 6 7 8

However, in our hands, the reaction of benzoquinone (**5**) with isoprene (**6**) afforded[11] 5,8-dihydro-1,4-dihydroxy-6-methylnaphthalene (**9**) and the two isomeric octahydroanthraquinones (**10**, **11**) [from isoprene (**6**) reacting with *both* C=C bonds of benzoquinone (**5**)] (Scheme 2). Herein are described the syntheses of the four anthraquinones (**1** - **4**) with full experimental details.

Scheme 2

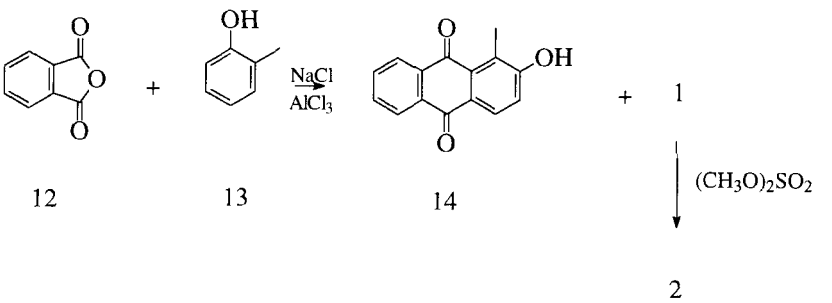


9

10 A = Me; B = H
11 A = H; B = Me

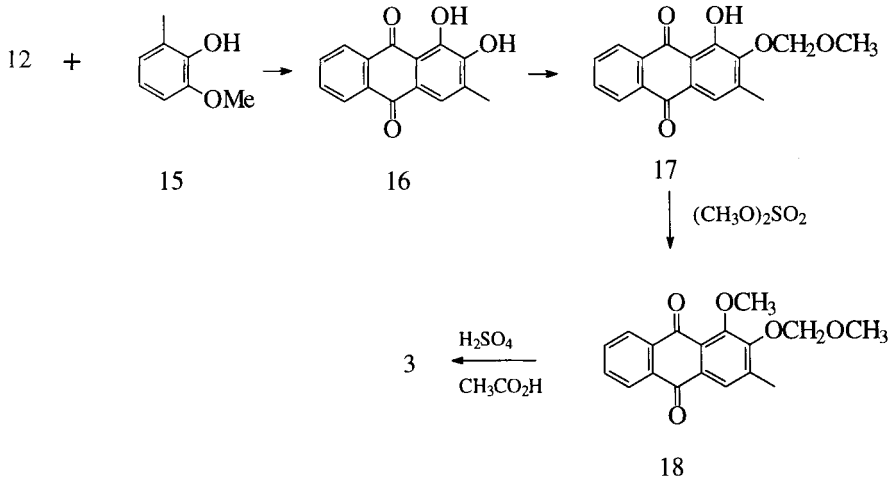
Reaction of phthalic anhydride (**12**) with *o*-cresol (**13**) in the presence of sodium chloride and aluminium trichloride afforded 2-hydroxy-3-methyl-9,10-anthraquinone (**1**) in good yield. (The 2-hydroxy-1-methyl isomer (**14**) was also isolated from this reaction.) Methylation of phenol (**1**) with dimethyl sulfate gave 2-methoxy-3-methyl-9,10-anthraquinone (**2**) in 60% yield (Scheme 3).

Scheme 3



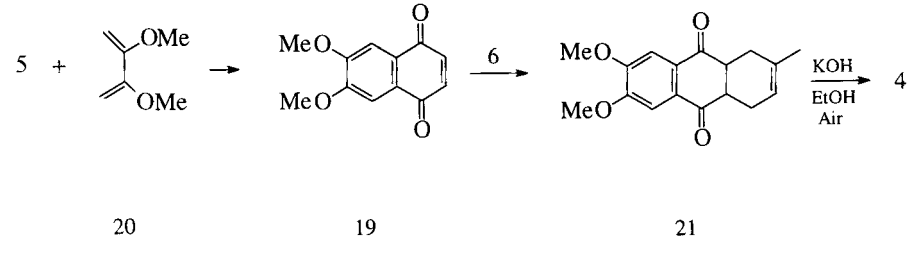
Friedel-Crafts reaction of phthalic anhydride (**12**) with 2-hydroxy-3-methoxytoluene (**15**) (prepared from 2,3-dimethoxytoluene) provided 1,2-dihydroxy-3-methyl-9,10-anthraquinone (**16**) in moderate yield. Selective protection of the 2-hydroxy function of diphenol (**16**) as the methoxymethyl ether (**17**) followed by methylation with dimethyl sulfate furnished the protected anthraquinone (**18**) in high yield. Deprotection of the methoxymethyl group from anthraquinone (**17**) using a mixture of sulphuric and acetic acids yielded the desired anthraquinone (**3**) (66% from **16**.) (Scheme 4).

Scheme 4



6,7-Dimethoxy-1,4-naphthoquinone (**19**) was prepared in excellent yield by the Diels-Alder reaction of benzoquinone (**5**) with 2,3-dimethoxy-1,3-butadiene (**20**) in air. Heating a solution of naphthoquinone (**19**) in ethanol containing isoprene (**6**) in a sealed tube at 100°C afforded the tetrahydroanthraquinone (**21**) in quantitative yield. Aerial oxidation of **21** in ethanolic potassium hydroxide provided the required 2,3-dimethoxy-6-methyl-9,10-anthraquinone (**4**) in high yield (Scheme 5).

Scheme 5



The four anthraquinones (**1** - **4**) (previously isolated[8] from *H. diffusa*) and anthraquinone (**14**) were tested for cytotoxicity in K562 human leukaemia, KB human cervical and P388 murine leukaemia cell lines. All five compounds (**1** - **4**, **14**) showed ID_{50}s (the concentration required to cause a 50% decrease in cell growth) greater than 17 μM. The cytotoxicities of these anthraquinones (**1** - **4**, **14**) are considerably lower than that of the anthracycline daunorubicin (ID_{50} = 19 nM, P388).

EXPERIMENTAL

Chemicals

Proton spectra were determined on Bruker AC 300 NMR spectrometer in deuterochloroform unless stated otherwise. Chemical shifts are expressed in δ values relative to tetramethylsilane. Infra-red spectra were recorded on a Mattson 1000 FTIR spectrometer. Electron impact mass spectra were determined on a VG Trio 2 mass spectrometer at an ionising energy of 70 eV. For chemical ionisation ammonia was used as the reagent gas.

6,7-Dimethoxy-1,4-naphthoquinone (**19**)

A solution of 1,4-benzoquinone (sublimed, 0.5 g, 5 mmol) and 2,3-dimethoxy-1,3-butadiene (**20**) (0.61 ml, 5 mmol) in dry ethanol (3 ml) was heated under reflux for 24 h. T.l.c. showed that the reaction had finished. The solvent was

evaporated under reduced pressure. Flash column chromatography (petrol:EtOAc 4:1) and recrystallisation from ethanol afforded the title compound (**19**) as orange needles (0.25 g, 33%). mp: 237-8°C (lit.[12] mp: 236-7°C). (Found: C, 66.15; H, 4.45. Calculated for $C_{12}H_{10}O_4$ C, 66.05; 4.62%). R_f: 0.32 (petrol:EtOAc 4:1); ν_{max} (KBr)/cm^{-1}: 1654 (C=O), 1610 (C=C); *m/z* (EI): 218 (M^+, 100%), *m/z* (CI): 236 ($M + NH_4^+$, 40), 219 (M + H, 100%).

2,3-Dimethoxy-6-methyl-5,8,8a,10a-tetrahydroanthraquinone (**21**)

A solution of 6,7-dimethoxynaphthoquinone (**19**) (0.654 g, 3 mmol) and isoprene (0.6 ml, 6 mmol) in dry ethanol (2 ml) were sealed in a glass tube (Pyrex, 15 ml) and heated at 100°C for 24 h. Evaporation of the solvent followed by recrystallisation from ethanol afforded the title anthraquinone (**21**) as light yellow crystals (0.55 g, 64%). mp: 190-1°C. Rf: 0.38 (petrol:EtOAc 1:1). ν_{max} (KBr)/cm^{-1}: 1673 (C=O); *m/z* (EI): 286 (M^+, 73), 271 (M - CH_3, 93), 258 (M - CO, 100%).

2,3-Dimethoxy-6-methylanthraquinone (**4**)

Air was bubbled through a gently warmed solution of 2,3-dimethoxy-6-methyl-5,5a,8,9a-tetrahydroanthraquinone (**21**) (0.54 g, 1.89 mmol) in 5% KOH in ethanol (20 ml) for 2 h. The initial dark brown colour changed to bright yellow. The precipitated yellow solid was filtered off and washed successively with water (10 ml), methanol (2 ml) and diethyl ether (1 ml). The product was dried in air and

recrystallised from acetic acid to give the title anthraquinone (**4**) as bright yellow crystals (0.38 g, 71%). mp: 231-2°C (lit.[13] mp: 237-8°C). Rf: 0.41 (petrol:EtOAc 1:1); ν_{max} (KBr)/cm^{-1}: 1674 (C=O); δ_H: 2.59 (3 H, s, Ar-Me); 4.11 (6 H, s, 2 x OMe); 7.6 (1 H, dd, J = 8, 2 Hz, 7-H); 7.76 (2 H, s, 1,4-H); 8.11 (1 H, s, 5-H); 8.21 (1 H, d, J = 8 Hz, 8-H); *m/z* (EI): 282 (M^+, 100%), 267 (M - Me, 11).

2-Hydroxy-3-methoxytoluene (**15**)

Trimethylsilyl iodide (5 ml, 35 mmol) was added slowly to a solution of 2,3-dimethoxytoluene (5 g, 32.9 mmol) in $CHCl_3$ (16 ml) under argon and the mixture stirred at room temperature for 48 h. To the above mixture were added methanol (160 ml) and brine (320 ml). The mixture was extracted with dichloromethane (3 x 200 ml) and the extracts washed with aqueous sodium bisulfite (10%, 3 x 200 ml), brine (2 x 200 ml) and dried over $MgSO_4$. Evaporation of the solvent followed by flash chromatography (petrol:EtOAc 20:1) afforded the title phenol (**15**) as a colourless oil, which crystallised on cooling as colourless needles (4.4 g, 97%). mp: 41-41.5°C (lit.[14] mp: 39-41°C). R_f: 0.23 (petrol:EtOAc 10:1); λ_{max} (MeOH)/nm: 275, 220 (275 shifted to 280 on addition of dilute aqueous NaOH); *m/z* (EI): 138 (M^+, 100%).

1,2-Dihydroxy-3-methylanthraquinone (**16**)

Phthalic anhydride (**12**) (4.88 g, 32.9 mmol) and 2-hydroxy-3-methoxytoluene (**15**) (4.5 g, 33 mmol) were mixed well and introduced into a melt of $AlCl_3$ (50

g) and NaCl (10 g) at 120°C (external temp.) The mixture was heated with stirring for 45 min at 165°C. After cooling, the deep red melt was decomposed by the addition of a mixture of ice and hydrochloric acid. The acidic mixture was briefly heated under reflux and the deep green residue extracted with ethyl acetate (5 x 500 ml). Flash chromatography (petrol:EtOAc 5:2) of the organic extract and recrystallisation from ethanol afforded the title anthraquinone (**16**) as orange crystals (2.67 g, 32 %). mp: 243-4°C (lit.[15] mp: 249-50°C.) (Found: C, 70.9; H, 4.0. Calculated for $C_{15}H_{10}O_4$ C, 70.9; H, 4.0%). R_f: 0.27 (petrol:EtOAc 5:2); δ_H: 2.45 (3 H, s, Ar-Me), 6.34 (1 H, s, OH exchanges with D_2O), 7.76 (1 H, s, 4-H), 7.78 - 7.89 (2 H, m, 6,7-Hs), 8.27 - 8.39 (2 H, m, 5,8-Hs), 12.80 (1 H, s, OH exchanges with D_2O).

1-Hydroxy-2-methoxymethoxy-3-methylanthraquinone (**17**)

To a solution of 1,2-dihydroxy-3-methylanthraquinone (**16**) (0.254 g, 1 mmol), dry K_2CO_3 (70 mg, 0.5 mmol) in dry acetone (10 ml) under nitrogen was slowly added chloromethylmethyl ether (38 µl, 0.5 mmol). After stirring at room temperature for 48 h the solvent was removed under reduced pressure. Flash chromatography (petrol:EtOAc 4:1) of the crude product followed by recrystallisation from ethanol yielded the title compound (**17**) as orange crystals (75 mg, 38%). mp: 127-8°C (lit.[10] mp: 108-110°C.) (Found: C, 68.30; H, 4.76. $C_{17}H_{14}O_5$ requires C, 68.49; H, 4.73%). R_f: 0.35 (petrol:EtOAc 4:1); ν_{max}

(KBr)/cm^{-1}: 1672 (C=O); λ_{max} (MeOH)/nm: 409, 264 (409 nm shifted to 510 and 264 to 252 on addition of dilute aqueous NaOH); δ_H: 2.42 (3 H, s, Ar-Me), 3.60 (3 H, s, $OCH_2OC\underline{H}_3$), 5.35 (2 H, s, $OC\underline{H}_2OMe$), 7.71 (1 H, s, 4-H), 7.73 - 7.82 (2 H, m, 6,7-Hs), 8.25 - 8.35 (2 H, m, 5,8-Hs), 12.94 (1 H, s, OH exchanges with D_2O). *m/z* (EI): 298 (M^+, 8%); 45 (100).

1-Methoxy-2-methoxymethoxy-3-methylanthraquinone (**18**)

To a solution of 1-hydroxy-2-methoxymethoxy-3-methylanthraquinone (**17**) (70 mg, 0.235 mmol) and K_2CO_3 (33 mg, 0.236 mmol) in dry acetone (10 ml) under nitrogen was added dimethylsulfate (44.4 µl, 0.47 mmol). The solution was stirred at room temperature for 60 h and after standing for further 5 h the reaction was quenched by addition of water (15 ml). The yellow solid formed was collected by filtration, washed with water (10 ml) and ethanol (2 ml). Recrystallisation of the crude product from ethanol afforded the title anthraquinone (**18**) as bright yellow needles (63 mg, 86%). mp: 141-2°C (lit.[10] mp: 123-5°C). (Found: C, 68.3; H, 4.8. $C_{17}H_{14}O_5$ requires C, 68.5; H, 4.7%). Rf: 0.24 (petrol:EtOAc 4:1). ν_{max} (KBr)/cm^{-1}: 1676 (C=O); λ_{max} (MeOH)/nm: 338, 263 and 205 (no shift on addition of dilute aqueous NaOH); δ_H: 2.5 (3 H, s, Ar-$C\underline{H}_3$), 3.66 (3 H, s, Ar-O$\underline{Me}$), 4.1 (3 H, s, $OCH_2OC\underline{H}_3$), 5.33 (2 H, s, $OC\underline{H}_2OMe$), 7.75 -7.85 (2 H, m, 6,7-Hs), 8.05 (1 H, s, 4-H), 8.25 - 8.32 (2 H, m, 5,8-Hs). *m/z* (EI): 312 (M^+, 6%), 281 (M - OMe, 13), 267 (M - CH_2OCH_3, 37), 45 (100).

2-Hydroxy-1-methoxy-3-methylanthraquinone (**3**)

1-Methoxy-2-methoxymethoxy-3-methylanthraquinone (**18**) (50 mg, 0.16 mmol) was dissolved in acetic acid (5 ml) containing a few drops of 1 M H_2SO_4 and the solution warmed at 70-80°C for 24 h. The solution was diluted with water (10 ml) and the solvent mixture was removed under reduced pressure. The crude product was extracted with $CHCl_3$ (3 x 10 ml), washed with water (2 x 10 ml) and brine (10 ml), and dried ($MgSO_4$). Concentration of the organic solution afforded the title anthraquinone (**3**) as bright yellow crystals (35 mg, 81%). mp: 220-2°C (ethanol) (lit.[10] mp: 220-2°C). R_f: 0.13 (petrol:EtOAc 4:1). ν_{max} (KBr)/cm^{-1}: 3200-3400 (OH), 1670 (C=O) and 1589. λ_{max} (MeOH)/nm: 384, 319 and 275 (384 shifted to 513, 319 disappeared and 275 to 254 on addition of dilute aqueous NaOH); δ_H: 2.42 (3 H, s, Ar-Me), 4.05 (3 H, s, Ar-OMe), 6.78 (1 H, s, OH exchanges with D_2O), 7.73 - 7.82 (2 H, m, 6,7-Hs), 8.0 (1 H, s, 4-H), 8.2 - 8.3 (2 H, m, 5,8-Hs). *m/z* (EI): 268 (M^+, 68), 250 (M - H_2O, 91), 222 (M - Me - OMe, 100%).

2-Hydroxy-3-methylanthraquinone (**1**) and *2-hydroxy-1-methylanthraquinone* (**14**)

Phthalic anhydride (**12**) (6.6 g, 44.6 mmol) and *o*-cresol (4.4 g, 40.7 mmol) were introduced into a melt of aluminum chloride (60 g, 450 mmol) and sodium chloride (12 g, 205 mmol) at 120-130°C (external temperature). The mixture was heated with stirring for 3 h at 165°C. After cooling, the deep red melt was decomposed by addition of a mixture of ice and hydrochloric acid and the residue

filtered. The deep green residue was heated under reflux with saturated aqueous sodium carbonate (500 ml). The solution was cooled and the precipitated 1-hydroxy-2-methylanthraquinone [0.12 g, 1%; R_f: 0.49 (petrol:EtOAc)] was filtered off and the filtrate saturated with carbon dioxide. Flash chromatography (petrol:EtOAc 5:2) of the filtrate followed by recrystallisation from ethanol afforded the 3-methyl isomer (**1**) as an orange powder (2.4 g, 23%). mp(d): 303-4°C (lit.[16] mp: 302°C). R_f: 0.25 (petrol:EtOAc 5:2); δ_H (DMSO-d_6): 2.40 (3 H, s, Me), 7.66 (1 H, s, 1-H), 7.91 - 8.03 (2 H, m, 6,7-Hs), 8.07 (1 H, s, 4-H), 8.20 - 8.30 (2 H, m, 5,8-Hs), 11.15 (1 H, s, OH). Concentration of the above mother liquid and recrystallization from ethanol gave 2-hydroxy-1-methylanthraquinone (**14**) as a green-yellow powder (1.6 g, 15%). mp: 244-5°C (lit.[16] mp: 238°C, from benzene). R_f: 0.20 (petrol:EtOAc 5:2). δ_H (DMSO-d_6): 2.69 (3 H, s, Me), 7.38 (1 H, d, J = 8.5 Hz, 3-H), 7.91 - 8.02 (2 H, m, 6,7-Hs), 8.14 (1 H, d, J = 8.5 Hz, 4-H), 8.18 - 8.28 (2 H, m, 5,8-Hs), 11.10 (1 H, s, OH). *m/z* (EI): 238 (M^+, 100%).

2-Methoxy-3-methylanthraquinone (**2**)

To a solution of 2-hydroxy-3-methylanthraquinone (**1**) (238 mg, 1 mmol) and K_2CO_3 (140 mg, 1 mmol) in acetone (10 ml) was added dimethyl sulfate (189 µl, 2 mmol). The solution was stirred at room temperature for 60 h and diluted with water (15 ml). The greenish yellow precipitate was filtered, washed with water (10 ml) and ethanol (5 ml). Recrystallisation of the crude product from acetic

acid afforded the title ether (**2**) as greenish yellow needles (150 mg, 60%). mp: 199-200°C (lit.[16] mp: 197°C). R_f: 0.32 (petrol:EtOAc 4:1); ν_{max} (KBr)/cm^{-1}): 1670 (C=O); λ_{max} (MeOH)/nm: 331, 274, 245, 240 and 205 (no shift on addition of dilute aqueous NaOH); δ_H: 2.42 (3 H, s, Ar-Me), 4.08 (3 H, s, OMe), 7.7 (1 H, s, 1-H), 7.75 - 7.88 (2 H, m, 6,7-Hs), 8.12 (1 H, s, 4-H), 8.27 - 8.4 (2 H, m, 5,8-Hs). *m/z* (EI): 252 (M^+, 100%), 237 (M - Me, 10), 223 (22).

Cytotoxicity Experiments

Cytotoxicity testing was performed as previously described.[17]

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