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ANTHRAQUINONES IN CALLUS CULTURES OF *CINCHONA LEDGERIANA*

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Key Word Index—*Cinchona ledgeriana*, Rubiaceae, callus cultures, anthraquinones, ^{1}H NMR

Abstract—From callus cultures of *Cinchona ledgeriana* seven known anthraquinones, purpurin, anthragallol-1,2-dimethylether, anthragallol-1,3-dimethylether, rubiadin, 1-hydroxy-2-hydroxymethylanthraquinone, 1-hydroxy-2-methylanthraquinone and morindone-5-methylether (or 1,7-dihydroxy-8-methoxy-2-methylanthraquinone), and eight new anthraquinones, 5,6-dimethoxy-1-(or -4-)hydroxy-2-(or -3-)hydroxymethylanthraquinone, 5-methoxy-2-(or -3-)methyl-1,4,6-trihydroxyanthraquinone, 2-hydroxy-1,3,4-trimethoxyanthraquinone, 4-methoxy-1,3,5-trihydroxyanthraquinone, 1,4-dimethoxy-2,3-methylenedioxyanthraquinone, 1,3-dihydroxy-4-methoxyanthraquinone, 1,3-dihydroxy-2,5-dimethoxyanthraquinone and 2,5-(or 3,5-)dihydroxy-1,3,4-(or -1,2,4-)trimethoxyanthraquinone have been isolated

INTRODUCTION

Many species of the Rubiaceae family are known to contain substantial amounts of anthraquinones both free and as glycosides [1] From *Rubia cordifolia* Tessier and co-workers [2–4] isolated and identified ten anthraquinones, and from *Galium album* Kuiper and Labadie [5] also isolated ten anthraquinones Tissue cultures of Rubiaceae species have also been shown to contain anthraquinones Inoue and co-workers [6] isolated from cell suspension cultures of *Morinda citrifolia* 12 anthraquinones, both aglucones and glycosides Until now only one report seems to have appeared in literature indicating that the genus *Cinchona* contains anthraquinones Covello and co-workers [7] reported on the occurrence of anthraquinones in 'Chinae Cortex' without, however, identifying the individual compounds

In a previous study, we reported on the isolation and identification (by means of TLC) of anthraquinone aglucones from a chloroform extract of callus tissue of *Cinchona ledgeriana* [8] Five anthraquinones were then identified alizarin, alizarin-1-methylether, rubiadin, 1,8-dihydroxyanthraquinone and 1-hydroxy-2-hydroxymethylanthraquinone In the present study, we report the isolation and identification of the anthraquinones present in a chloroform extract of *C ledgeriana* callus tissue Fifteen anthraquinones were isolated by means of preparative TLC and identified by means of spectroscopic methods Seven of the anthraquinones had previously been found in Rubiaceae species, eight of them were new

RESULTS AND DISCUSSION

Fifteen pure compounds were isolated in sufficient amounts for further spectroscopic characterization (Table 1, Fig 1) Four of them were known compounds, which were identified by comparison of their spectral data with those reported in the literature purpurin [1, 5], rubiadin [4–6], 1-hydroxy-2-hydroxymethylanthraquinone [5] and 1-hydroxy-2-methylanthraquinone [2] The other ones were identified on the basis of their spectroscopic properties Three of the anthraquinones which previously had been identified in the same cell line by means of TLC by Mulder-Krieger *et al* [8] could not, however, be detected in this study alizarin, alizarin-1-methylether and 1,8-dihydroxyanthraquinone The reason might be that the anthraquinones in the previous study were identified in an extract meant to contain no other compounds than alkaloids, whereas in the present study a purified anthraquinone extract was used

Anthragallol-1,2-dimethylether (2)

This yellow compound has an M^+ at *m/z* 284 Its ^{1}H NMR spectrum shows two three-proton singlets due to methoxyl groups at *ca* δ4, one one-proton singlet at δ7 60 and a pattern characteristic of an unsubstituted A-ring multiplets at δ7 7 and 8 2 The IR spectrum shows an absorption band at 3420 cm^{-1}, characteristic of a free hydroxyl group and only one absorption band in the region between 1600 and 1700 cm^{-1}, at 1660 cm^{-1}, indicating that both carbonyl groups are unchelated [1] The MS shows a peak of rather small intensity due to the loss of water from the molecular ion, indicating that at least one methoxyl group is in the 1- or 4-position [9] The value for the shift of the proton from the substituted C-ring is more likely to be due to a proton at C-4 rather than at C-3 and shows good agreement with the shift for H-4 in anthragallol-1,2,3-trimethylether [10] From the melting point (228–232°) [1, 11] and from NOE difference experiments it was finally concluded that the compound isolated from band 5 1 is 1,2-dimethoxy-3-hydroxyanthraquinone (anthragallol-1,2-dimethylether)

Anthragallol-1,3-dimethylether (3)

This yellow anthraquinone has an M^+ at *m/z* 284 Its ^{1}H NMR spectrum shows two three-proton singlets at *ca* δ4, one one-proton singlet at δ7 72 and the two multiplets at δ7 7 and 8 2 characteristic of an unsubstituted A-ring

Table 1 R_f values and colours of the anthraquinones isolated from *C ledgeriana*

Compound	R_f		Colour				
	S1*	S2	Daylight	UV_{254}	UV_{366}	NH_3 vapour	NaOH†
1	0 00	0 00	red–purple		red	red–purple	red–purple
2	0 41	0 25	yellow		red	orange	orange
3	0 41	0 29	dark yellow		orange	orange	red
4	0 58	0 37	yellow		brown	brown	red
5	0 71	0 31	bright yellow	red	orange	red	red
6	0 79	0 65	yellow	red–brown	orange	yellow	red
7	0 35	0 35	dark yellow	red–brown	yellow–orange	red–purple	red–purple
8	0 69	0 28	orange–yellow	red	orange	red	red
9	0 35	0 36	orange–red	red–brown	yellow–orange	red–purple	red–purple
10	0 29	0 25	dark yellow		red	orange	orange
11	0 23	0 33	orange		red	red–brown	red–purple
12	0 79	0 45	bright yellow		bright yellow	yellow	yellow
13	0 28	0 33	orange		red	red–brown	red–purple
14	0 33	0 30	orange–red	red–brown	orange	red–brown	purple
15	0 25	0 27	orange		orange	red	red

*S1, Silica gel 60 (precoated TLC plate), chloroform–methanol–ammonia 25% (85 14 1) saturated, developing distance 15 cm S2, Silica gel 60 (precoated TLC plate), toluene–methanol (9 1) saturated, developing distance 15 cm

†Colours with NaOH were obtained by spraying the chromatograms with a solution of NaOH in methanol (5% w/v)

The IR spectrum shows an absorption band at 3420 cm^{-1} due to a free hydroxyl group, and only one absorption band in the region between 1600 and 1700 cm^{-1}, at 1675 cm^{-1}, indicating that both carbonyl groups are unchelated The value for the shift of the proton from the substituted C-ring is more likely to be due to a proton at C-4 rather than at C-3 Since this compound differs from anthragallol-1,2-dimethylether it is therefore concluded that this anthraquinone is 1,3-dimethoxy-2-hydroxyanthraquinone (anthragallol-1,3-dimethylether)

1,6-Dihydroxy-5-methoxy-2-methylanthraquinone or 1,7-*dihydroxy-8-methoxy-2-methylanthraquinone* (**7**)

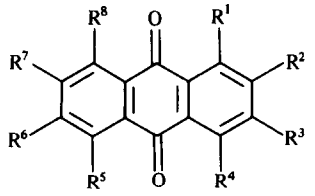
This orange–yellow anthraquinone derivative has an M^+ at *m/z* 284 The ^1H NMR spectrum shows a three-proton singlet at δ2 38 due to a methyl group, a three-proton singlet at δ4 02 due to a methoxyl group and a one-proton singlet at δ13 1 due to a chelated hydroxyl group Four aromatic protons appear in the ^1H NMR spectrum as two pairs of doublets The IR spectrum shows an absorption band at 3420 cm^{-1} due to a free hydroxyl group and in the region between 1600 and 1700 cm^{-1} two absorption bands due to one free and one chelated carbonyl group (at 1660 and 1625 cm^{-1} respectively) The UV spectrum recorded in MeOH–OH^- shows a maximum at 500 nm from which it can be concluded that the two hydroxyl groups are not in the same ring [12] On the basis of this spectral data and from biogenetic considerations [13], it is concluded that this anthraquinone derivative is either 1,6-dihydroxy-5-methoxy-2-methylanthraquinone or 1,7-dihydroxy-8-methoxy-2-methylanthraquinone The amount isolated did not allow us to discriminate between these two possible structures by means of chemical reactions

5,6-Dimethoxy-1-hydroxy-2-hydroxymethylanthraquinone or 5,6-*dimethoxy-4-hydroxy-3-hydroxymethylanthraquinone* (**8**)

This orange–yellow anthraquinone derivative has an M^+ at *m/z* 314 Its ^1H NMR spectrum shows two three-proton singlets at δ3 83 and 3 96 due to two methoxyl groups, a two proton singlet at δ4 63 due to a hydroxymethyl group (cf anthraquinone **5**), two pairs of doublets in the aromatic region and a one-proton singlet due to a chelated hydroxyl group at δ13 1 The MS shows an $[M - H_2O]^+$ peak at *m/z* 296 The UV spectrum recorded in MeOH–OH^- shows a maximum at 480 nm On the basis of this spectral data it is concluded that this compound is either 5,6-dimethoxy-1-hydroxy-2-hydroxymethylanthraquinone or 5,6-dimethoxy-4-hydroxy-3-hydroxymethylanthraquinone The amount isolated did not allow us to discriminate between these two possible structures by means of chemical reactions

5-Methoxy-2-methyl-1,4,6-trihydroxyanthraquinone or 5-*methoxy-3-methyl-1,4,6-trihydroxyanthraquinone* (**9**)

This red anthraquinone derivative has an M^+ at *m/z* 300 Its ^1H NMR spectrum shows a three-proton singlet at δ2 36 due to a methyl group, a three-proton singlet at δ4 04 due to a methoxyl group and two one-proton singlets at δ13 1 and 13 4 due to two chelated hydroxyl groups The ^1H NMR spectrum shows in the aromatic region a one-proton singlet at δ7 18 and a pair of doublets at δ7 4 and 8 2 (J = 8 Hz) The IR spectrum shows an absorption band at *ca* 3400 cm^{-1} due to a free hydroxyl group and only one absorption band in the region between 1600 and 1700 cm^{-1}, at 1610 cm^{-1}, indicating that both carbonyl groups are chelated The maximum in the UV spectrum recorded in MeOH–OH^-



	R^1	R^2	R^3	R^4	R^5	R^6	R^7	R^8
Purpurin (**1**)	OH	OH	H	OH	H	H	H	H
Anthragallol-1, 2-dimethylether (**2**)	OMe	OMe	OH	H	H	H	H	H
Anthragallol-1, 3-dimethylether (**3**)	OMe	OH	OMe	H	H	H	H	H
Rubiadin (**4**)	OH	Me	OH	H	H	H	H	H
1 Hydroxy-2-hydroxymethyl A* (**5**)	OH	CH_2OH	H	H	H	H	H	H
1-Hydroxy 2-methyl A (**6**)	OH	Me	H	H	H	H	H	H
Morindone-5-methylether or 1,7 dihydroxy 8-methoxy-2-methyl A (**7**)	OH	Me	H	H	OMe or H	OH or H	H or OH	H or OMe
5,6 Dimethoxy-1 (or-4-) hydroxy -2-(or 3) hydroxymethyl A (**8**)	OH or H	CH_2OH or H	H or CH_2OH	H or OH	OMe	OMe	H	H
5-Methoxy-2-(or 3-) methyl-1,4,6 -trihydroxy A (**9**)	OH	Me or H	H or Me	OH	OMe	OH	H	H
2 Hydroxy-1,3,4-trimethoxy A (**10**)	OMe	OH	OMe	OMe	H	H	H	H
4-Methoxy-1,3 5 trihydroxy A (**11**)	OH	H	OH	OMe	OH	H	H	H
1,4-Dimethoxy-2,3-methylenedioxy A (**12**)	OMe	$O-CH_2-O$		OMe	H	H	H	H
1,3-Dihydroxy 4 methoxy A (**13**)	OH	H	OH	OMe	H	H	H	H
1,3-Dihydroxy-2,5-dimethoxy A (**14**)	OH	OMe	OH	H	OMe	H	H	H
2,5-(or 3,5-) Dihydroxy-1,3,4- or (or-1,2,4-) trimethoxy A (**15**)	OMe	OH or OMe	OMe or OH	OMe	OH	H	H	H

*A = anthraquinone

Fig 1 Structures of the anthraquinones isolated from callus culture of *C ledgeriana*

is at 525 nm On the basis of this spectral data no conclusion could be drawn about the substitution pattern of this compound, therefore some NOE difference experiments were performed From these experiments certainty about the position of the aromatic proton from the C-ring could be obtained, but from the NOEs observed it was concluded that this compound, though one spot on TLC, is probably a mixture of the two possible isomers (5-OMe, 6-OH and 8-OMe, 7-OH)

2-Hydroxy-1,3,4-*trimethoxyanthraquinone* (**10**)

This orange anthraquinone has an M^+ at *m/z* 314 The ^1H NMR spectrum shows three three-proton singlets due to methoxyl groups at δ3 99, 4 02 and 4 10, and two multiplets at δ7 7 and 8 2 characteristic of an unsubstituted A-ring The IR spectrum shows an absorption band due to a free hydroxyl group at 3420 cm^{-1} This means that ring C is fully substituted with one hydroxyl group at C-2 or C-3 and three methoxyl groups This is in good agreement with the maximum in the UV spectrum recorded in MeOH–OH^- at 476 nm and the presence in the MS of an $[M-H_2O]^+$ peak at *m/z* 296 On the basis of this spectral data, it is therefore concluded that this compound is 2-hydroxy-1,3,4-trimethoxyanthraquinone

4-Methoxy-1,3,5-*trihydroxyanthraquinone* (**11**)

This anthraquinone has an M^+ at *m/z* 286 The ^1H NMR spectrum shows a three-proton singlet due to a methoxyl group at δ4 13, a one-proton singlet at δ7 46 and two doublets (δ7 26 and 7 82) and one triplet (δ7 63) characteristic of a monosubstituted A-ring The IR spectrum shows absorption bands due to a free hydroxyl group (3420 cm^{-1}) and due to two chelated carbonyl groups (1630 cm^{-1}) The MS shows a prominent $[M-H_2O]^+$ peak at *m/z* 268 (90% of base peak), indicative of a peri-positioned methoxyl group The UV spectrum

recorded in MeOH–OH^- shows a maximum at 485 nm which is characteristic for a 1,3-dihydroxyanthraquinone [12] Combining this spectral data leads us to the conclusion that this compound is 4-methoxy-1,3,5-trihydroxyanthraquinone

1,4-*Dimethoxy*-2,3-*methylenedioxyanthraquinone* (**12**)

This yellow anthraquinone derivative has an M^+ at *m/z* 312 The ^1H NMR spectrum shows a six-proton singlet at δ4 07 due to two methoxyl groups, a two-proton singlet at δ6 18 and two multiplets at δ7 7 and 8 2 characteristic of an unsubstituted A-ring A two-proton singlet at δ6 18 is due to a methylenedioxy group in the molecule [14] The MS shows an $[M-H_2O]^+$ peak at *m/z* 294 On the basis of this spectral data it is concluded that this anthraquinone is 1,4-dimethoxy-2,3-methylenedioxyanthraquinone For this new anthraquinone we propose the trivial name ledgerquinone

1,3-*Dihydroxy*-4-*methoxyanthraquinone* (**13**)

This anthraquinone has an M^+ at *m/z* 270 The ^1H NMR spectrum shows a three-proton singlet due to a methoxyl group at δ4 14, a one-proton singlet at δ7 45 and the two multiplets at δ7 7 and 8 2 characteristic of an unsubstituted A-ring The IR spectrum shows absorption bands due to a free hydroxyl group (3400 cm^{-1}), a free carbonyl group (1660 cm^{-1}) and a chelated carbonyl group (1630 cm^{-1}) The MS shows a prominent $[M-H_2O]^+$ peak at *m/z* 252 (75% of base peak), from which it can be concluded that the methoxyl group is at C-1 or C-4 The UV spectrum recorded in MeOH–OH^- shows a maximum at 485 nm which is characteristic of a 1,3-dihydroxyanthraquinone From this spectral data it is concluded that this compound is 1,3-dihydroxy-4-methoxyanthraquinone (equivalent to purpurin-1-methylether)

1,3-*Dihydroxy*-2,5-*dimethoxyanthraquinone* (**14**)

This red anthraquinone derivative has an M^+ at *m/z* 300 The ^1H NMR spectrum shows two three-proton singlets at δ3 98 and 3 99 due to two methoxyl groups, two doublets with a long-range (*meta*) coupling and a triplet in the aromatic region characteristic of a mono-substituted A-ring A one-proton singlet (δ7 63) in the aromatic region indicates that the other ring is trisubstituted The IR spectrum shows an absorption band at 3420 cm^{-1} due to a free hydroxyl group and absorption bands at 1670 and 1630 cm^{-1} indicating that one of the carbonyl groups is chelated The UV spectrum recorded in MeOH–OH^- shows a maximum at 505 nm From this spectral data it can be concluded that this compound is either 1,3-dihydroxy-2,5-dimethoxyanthraquinone or 1,3-dihydroxy-2,8-dimethoxyanthraquinone To discriminate between these two structures the unknown compound was demethylated using HBr in pyridine as a demethylating agent The IR spectrum of the demethylated product showed only one absorption band in the region between 1600 and 1700 cm^{-1} at 1630 cm^{-1} indicating that after demethylation both carbonyl groups are chelated On the basis of this data it was concluded that this anthraquinone is 1,3-dihydroxy-2,5-dimethoxyanthraquinone

2,5-*Dihydroxy*-1,3,4-*trimethoxyanthraquinone or* 3,5-*dihydroxy*-1,2,4-*trimethoxyanthraquinone* (**15**)

This anthraquinone has an M^+ at *m/z* 330 The ^1H NMR spectrum shows three three-proton singlets due to methoxyl groups at *ca* δ4 and two doublets and one triplet in the aromatic region indicating that one ring is fully substituted while the other is monosubstituted The IR spectrum shows an absorption band due to a free hydroxyl group (3400 cm^{-1}) and absorption bands due to one free and one chelated carbonyl group at 1660 and 1630 cm^{-1} respectively The MS shows a small $[M-H_2O]^+$ peak at *m/z* 312 From the maximum in the UV spectrum recorded in MeOH–OH^- it can be concluded that the two hydroxyl groups are not in the same ring ($\lambda_{max}^{MeOH-OH^-}$ 1,2-di-OH-A = 575 nm, $\lambda_{max}^{MeOH-OH^-}$ 1,3-di-OH-A = 485 nm, $\lambda_{max}^{MeOH-OH^-}$ 1,4-di-OH-A = 560 nm) [12] From this spectral data it is concluded that this compound is either 2,5-dihydroxy-1,3,4-trimethoxyanthraquinone or 3,5-dihydroxy-1,2,4-trimethoxyanthraquinone The spectral data and the amount isolated did not allow us to discriminate between these two possible structures

The occurrence of the anthraquinones in the genus *Cinchona* gives chemotaxonomical proof for the relationship existing between this genus and the other genera of the Rubiaceae

EXPERIMENTAL

Biological material Callus cultures of *C ledgeriana* were grown on medium H and under the conditions described in a previous publication [15]

Extraction Ground, freeze-dried calli were mixed with 10% aq $NaHCO_3$ soln and extracted with $CHCl_3$ After evaporation of the solvent the residue was redissolved in Et_2O The ethereal soln was extracted with 1 M NaOH, which was then acidified with HCl and extracted with Et_2O This gave the final extract containing almost nothing but anthraquinone aglucones

Prep TLC Prior to development with the appropriate solvent system, the silica gel plates were developed with the same solvent system The solvent systems used were S_1 ($CHCl_3$–MeOH–25% NH_4OH, 85 14 1) and S_2 (toluene–MeOH, 9 1) in combination with either home-made 0 50 mm layer thickness TLC plates (Merck Kiesel gel PF 254) or 0 20 mm layer thickness TLC plates with preconcentrating zone (Macherey-Nagel SilGur UV 254), all in saturated chambers After detection of the anthraquinones in daylight, the various bands were scraped off and the anthraquinones eluted with mixtures of MeOH and $CHCl_3$, varying from 90% MeOH for the most polar compound to 90% $CHCl_3$ for the least polar compound Analytical TLC was performed using the same solvent systems as described above in combination with ready-made TLC plates (Merck Silica 60 F 254) Anthraquinones were detected by their colours in daylight, their fluorescence in UV light (254 and 366 nm), their colours after exposure to NH_3 vapour and their colours after spraying with a 5% soln of NaOH in MeOH EIMS were recorded on a Kratos M9/50 instrument using a direct inlet system and an ionizing energy of 70 eV 100 MHz ^1H NMR spectra were recorded on a Jeol PS-100 apparatus operating in the Fourier-transform mode 300 MHz ^1H NMR spectra were recorded on a Bruker WM 300 instrument Shifts are given in δ-values (ppm) relative to TMS IR spectra were determined either as KBr disc or as a soln in $CHCl_3$

Purpurin (**1**) MS (90°) *m/z* (rel int) 256 $[M]^+$ (30), 111 (35), 97 (50), 83 (50), 71 (75), 57 (100), UV-VIS λ_{max}^{MeOH} nm 218, 260, 280 (sh), 515

Anthragallol-1,2-*dimethylether* (**2**) MS (200°) *m/z* (rel int) 284 $[M]^+$ (95), 269 $[M-Me]^+$ (80), 266 $[M-H_2O]^+$ (5), 256 $[M-CO]^+$ (25), 83 (63), 55 (85), 43 (100), ^1H NMR (100 MHz,

$CDCl_3$) δ 3 99 (3H, *s*, OMe), 4 03 (3H, *s*, OMe), 7 60 (1H, *s*, H-4), 7 75 (2H, *m*, H-6, H-7), 8 25 (2H, *m*, H-5, H-8), UV-VIS λ_{max}^{MeOH} nm 238, 242, (sh), 279, 309, $\lambda_{max}^{MeOH-OH^-}$ nm 244, 311, 466, IR $\nu_{max}^{CHCl_3}$ cm^{-1} 3420, 2980, 1660, 1575 Mp (Kofler hot plate, uncorr) 228–232°

Anthragallol-1,3-*dimethylether* (**3**) MS (200°) *m/z* (rel int) 284 $[M]^+$ (39), 269 $[M-Me]^+$ (30), 266 $[M-H_2O]^+$ (5), 71 (60), 57 (100), 43 (90), ^{1}H NMR (300 MHz, $CDCl_3$) δ 3 99 (3H, *s*, OMe), 4 11 (3H, *s*, OMe), 7 72 (1H, *s*, H-4), 7 75 (2H, *m*, H-6, H-7), 8 26 (2H, *m*, H-5, H-8), UV-VIS λ_{max}^{MeOH} nm 220 (sh), 242 (sh), 278, 310 (sh), $\lambda_{max}^{MeOH-OH^-}$ nm 230 (sh), 246, 312, 490; IR ν_{max}^{KBr} cm^{-1} 3420, 1675, 1570

Rubiadin (**4**) The spectroscopic data agrees well with those in the literature [4–6]

1-*Hydroxy*-2-*hydroxymethylanthraquinone* (**5**) The spectroscopic data agrees well with those in the literature [5]

1-*Hydroxy*-2-*methylanthraquinone* (**6**) The spectroscopic data agrees well with those in the literature [2]

Morindone-5-*methylether or* 1,7-*dihydroxy*-8-*methoxy*-2-*methylanthraquinone* (**7**) MS (110°) *m/z* (rel int) 284 $[M]^+$ (100), 266 $[M-H_2O]^+$ (45), 238 (48), 60 (30), ^{1}H NMR (300 MHz, $CDCl_3$) δ 2 38 (3H, *s*, Me), 4 02 (3H, *s*, OMe), 7 35 (1H, *d*, *J* = 8 6 Hz, H-6 or H-7), 7 51 (1H, *d*, *J* = 7 8 Hz, H-3), 7 70 (1H, *d*, *J* = 7 8 Hz, H-4), 8 14 (1H, *d*, *J* = 8 6 Hz, H-8 or H-5), 13 1 (1H, *s*, OH), UV-VIS λ_{max}^{MeOH} nm 220, 271, 441, $\lambda_{max}^{MeOH-OH^-}$ nm 230 (sh), 242 (sh), 309, 500, IR ν_{max}^{KBr} cm^{-1} 3420, 2920, 2850, 1660, 1625

5,6-*Dimethoxy*-1-(*or* -4-)*hydroxy*-2-(*or* -3-)*hydroxymethylanthraquinone* (**8**) MS (115°) *m/z* (rel int) 314 $[M]^+$ (42), 296 $[M-H_2O]^+$ (8), 283 (100), 209 (52), 152 (34), ^{1}H NMR (300 MHz, $CDCl_3$) δ 3 83 (3H, *s*, OMe), 3 96 (3H, *s*, OMe), 4 63 (2H, *s*, CH_2OH), 7 57 (1H, *d*, *J* = 9 Hz, H-7), 7 65 (1H, *d*, *J* = 6 Hz, H-3 or H-2), 7 84 (1H, *d*, *J* = 6 Hz, H-4 or H-1), 8 11 (1H, *d*, *J* = 9 Hz, H-8), 13 10 (1H, *s*, OH), UV-VIS λ_{max}^{MeOH} nm 223, 240 (sh), 275 (sh), 395, $\lambda_{max}^{MeOH-OH^-}$ nm 235, 480

5-*Methoxy*-2-(*or* -3-)*methyl*-1,4,6-*trihydroxyanthraquinone* (**9**) MS (200°) *m/z* (rel int) 300 $[M]^+$ (40), 282 $[M-H_2O]^+$ (39), 254 $[282-CO]^+$ (20), 135 (25), 97 (31), 83 (63), 71 (60), 57 (100), ^{1}H NMR (100 MHz, $CDCl_3$) δ 2 36 (3H, *s*, Me), 4 04 (3H, *s*, OMe), 7 18 (1H, *s*, H-3 or H-2), 7 38 (1H, *d*, *J* = 8 Hz, H-7), 8 18 (1H, *d*, *J* = 8 Hz, H-8), 13 14 (1H, *s*, OH), 13 46 (1H, *s*, OH), UV-VIS λ_{max}^{MeOH} nm 222, 272, 457, $\lambda_{max}^{MeOH-OH^-}$ nm 240, 302, 525, IR $\nu_{max}^{CHCl_3}$ cm^{-1} 3420, 1610, 1565

2-*Hydroxy*-1,3,4-*trimethoxyanthraquinone* (**10**) MS (95°) *m/z* (rel int) 314 $[M]^+$ (100), 299 $[M-Me]^+$ (60), 296 $[M-H_2O]^+$ (8), 281 $[299-H_2O]^+$ (20), 271 (20), 256 (30), 211 (23), 157 (23), ^{1}H NMR (300 MHz, $CDCl_3$) δ 3 99 (3H, *s*, OMe), 4 02 (3H, *s*, OMe), 4 09 (3H, *s*, OMe), 7 72 (2H, *m*, H-6 H-7), 8 18 (2H, *m*, H-5, H-8), UV-VIS λ_{max}^{MeOH} nm 205, 240, 276, 366, $\lambda_{max}^{MeOH-OH^-}$ nm 251, 315, 476, IR ν_{max}^{KBr} cm^{-1} 3400, 1668, 1590

4-*Methoxy*-1,3,5-*trihydroxyanthraquinone* (**11**) MS (110°) *m/z* (rel int) 286 $[M]^+$ (100), 268 $[M-H_2O]^+$ (87), 257 $[M-CHO]^+$ (10), 243 (38), 212 (27), 180 (30), ^{1}H NMR (100 MHz, $CDCl_3$) δ 4 13 (3H, *s*, OMe), 7 26 (1H, *dd*, *J* = 9 Hz, *J* = 1 4 Hz, H-6), 7 46 (1H, *s*, H-2), 7 63 (1H, *t*, *J* = 8 Hz, H-7), 7 82 (1H, *dd*, *J* = 9 Hz, *J* = 1 4 Hz, H-8), 12 08 (1H, *s*, OH), 12 49 (1H, *s*, OH), UV-VIS λ_{max}^{MeOH} nm 279, 320, 425, 470 (sh), 485 (sh), $\lambda_{max}^{MeOH-OH^-}$ nm. 283, 315 (sh), 485, IR ν_{max}^{KBr} cm^{-1} 3420, 2920, 2860, 1720, 1630, 1470

1,4-*Dimethoxy*-2,3-*methylenedioxyanthraquinone* (**12**). MS (250°) *m/z* (rel int) 312 $[M]^+$ (48), 297 $[M-Me]^+$ (9), 294 $[M-H_2O]^+$ (5), 269 $[297-CO]^+$ (12), 254 (14), 85 (40), 71 (62), 57 (90), 43 (100); ^{1}H NMR (100 MHz, $CDCl_3$) δ 4 07 (6H, *s*, 2 × OMe), 6 18 (2H, *s*, OCH_2O), 7 70 (2H, *m*, H-6, H-7), 8 14 (2H, *m*, H-5, H-8), UV-VIS λ_{max}^{MeOH} nm 220 (sh), 241 (sh), 276, 355, $\lambda_{max}^{MeOH-OH^-}$ nm 220 (sh), 241 (sh), 276, 386

1,3-*Dihydroxy*-4-*methoxyanthraquinone* (**13**) MS (95°) *m/z* (rel int): 270 $[M]^+$ (100), 252 $[M-H_2O]^+$ (63), 241 $[M-CHO]^+$ (8), 227 (32), 196 (23), 85 (20), 71 (33), 57 (44), ^{1}H NMR (100 MHz, $CDCl_3$): δ 4 14 (3H, *s*, OMe), 7 45 (1H, *s*, H-2), 7 77 (2H, *m*, H-6, H-7), 8 26 (2H, *m*, H-5, H-8), UV-VIS λ_{max}^{MeOH} nm. 209, 240, 245 (sh), 284, 312, 415, 480 (sh); $\lambda_{max}^{MeOH-OH^-}$ nm 240, 245 (sh), 312, 485, IR ν_{max}^{KBr} cm^{-1} 3400, 2920, 2840, 1660, 1630, 1590

1,3-*Dihydroxy*-2,5-*dimethoxyanthraquinone* (**14**). MS (200°) *m/z* (rel int.) 300 $[M]^+$ (82), 285 $[M-Me]^+$ (88), 282 $[M-H_2O]^+$ (24), 270 $[285-CO]^+$ (40), 239 (35), 227 (43), 69 (45), 57 (70), 43 (100), ^{1}H NMR (300 MHz, DMSO-d_6): δ 3 98 (3H, *s*, OMe), 3 99 (3H, *s*, OMe), 7 25 (1H, *dd*, *J* = 8 Hz, *J* = 1 Hz, H-6), 7 60 (1H, *t*, *J* = 8 Hz, H-7), 7 63 (1H, *s*, H-4), 7 70 (1H, *dd*, *J* = 8 Hz, *J* = 1 Hz, H-8), 12.99 (1H, *s*, OH), UV-VIS λ_{max}^{MeOH} nm 220 (sh), 280, 310 (sh), 390; $\lambda_{max}^{MeOH-OH^-}$ nm 248, 313, 505, IR ν_{max}^{KBr} cm^{-1} 3420, 1670, 1630; IR after demethylation ν_{max}^{KBr} cm^{-1} 3420, 1630

2,5-(*or* 3,5-)*Dihydroxy*-1,3,4-(*or* -1,2,4-)*trimethoxyanthraquinone* (**15**). MS (110°) *m/z* (rel int) 330 $[M]^+$ (100), 315 $[M-Me]^+$ (60), 312 $[M-H_2O]^+$ (5), 297 $[315-H_2O]^+$ (20), 287 (22), 272 (24), 227 (20), 58 (23); ^{1}H NMR (300 MHz, $CDCl_3$) δ 3 99 (3H, *s*, OMe), 4 01 (3H, *s*, OMe), 4 10 (3H, *s*, OMe), 7 24 (1H, *d*, *J* = 7 8 Hz, H-6), 7 60 (1H, *t*, *J* = 7 8 Hz, *J* = 8 0 Hz, H-7), 7 71 (1H, *d*, *J* = 8 Hz, H-8), UV-VIS λ_{max}^{MeOH} nm 218, 276, 410; $\lambda_{max}^{MeOH-OH^-}$ nm. 253, 319, 500; IR ν_{max}^{KBr} cm^{-1} 3400, 2920, 2840, 1660, 1630, 1540

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