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NOVEL ANTHRAQUINONES FROM UNDIFFERENTIATED CELL CULTURES OF *GALIUM VERUM*

DEREK V. BANTHORPE* and JOHN J. WHITE†

Chemistry Department, University College London, London WC1H OAJ, U.K.

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Key Word Index—*Galium verum*; Rubiaceae; tissue culture; anthraquinones.

Abstract—Lines of callus of *Galium verum* established under a variety of culture conditions readily produced anthraquinones. Eight of the main pigments were purified, of which six were compounds new to the species. Six were fully identified as: 1,3-dihydroxy-2 methoxymethyl, 1,3-dimethoxy-2-hydroxy, 1,3-dihydroxy-2-acetoxy, 1-hydroxy-2-hydroxymethyl, 1,3-dihydroxy-2-methyl and 1-methoxy-2-hydroxyanthraquinones. The other two were provisionally identified as 1,3-dihydroxy-2-hydroxymethyl-6-methoxy and 1,6-dihydroxy-2-methylanthraquinones.

INTRODUCTION

Galium verum L. (Lady's bedstraw, Rubiaceae) has been widely used in folk medicine and as a food additive, and the aerial parts and roots contain yellow and red pigments, respectively, that have found use in dyeing [1, 2]. Several anthraquinones have been isolated from the latter tissue [3–5]. Anthraquinone production from cell cultures of *G. verum* has been optimized [6] using a spectrophotometric assay, but no compounds were identified. However, several anthraquinones have been characterized from cultures of other members of the Rubiaceae [7, 8], including other *Galium* species [9, 10]. The objective of the present study was to establish pigmented cultures of *G. verum* and to determine the pattern of the (presumed) anthraquinones that were accumulated.

RESULTS AND DISCUSSION

Callus was readily established from stem explants of *G verum* and after maintenance under a variety of culture conditions, i.e. sucrose (3–12% wt/v), photoperiod (continuous; 16 hr diurnal illumination) and growth regulators—namely GA_3 (2–10 mg l^{-1}), BAP (0.5–5 mg l^{-1}), NAA (0.1–2.0 mg l^{-1}), kinetin (0.2 mg l^{-1}), 2,4-D (1–2 mg l^{-1}) and casein (3 g l^{-1}) for five subcultures (cell cycles 7–28 days), the lines were assayed. All lines rapidly became intensely red or orange pigmented, although little colour was released into the medium. Eighteen lines were established that did not exhibit obvious differentiation (i.e. shoot or bud formation) and all appeared similar in morphology comprising essentially undifferentiated parenchyma-like tissue sometimes with signs of tracheid formation and secondary hardening. Increase of the sucrose content of the medium from 3% (the norm) to 12% w/v led to a study reduction in growth to *ca* 25% of the optimal, whereas the pigment accumulation (measured as alizarin equivalents) increased from 7 to 13 μmol g^{-1} FW. Such relationships have been reported for anthraquinone production in cell cultures of other species [6, 11–13]. Extracts of all culture lines showed similar profiles of products when examined by TLC with apparently the same six or so major compounds predominating. Consequently, it was presumed that a facile 'background' metabolism had been achieved and all extracts were pooled for work-up of the aglycones of the pigments. However, examination of this material by either TLC, gel-filtration or column chromatography using methods recommended for the separation of anthraquinones [14–16] led to extremely complex mixtures as adjudged by analytical HPLC, and an extensive scheme of purification using preparative-scale HPLC had to be undertaken to obtain products homogeneous (>98%) by the previous criterion. Preliminary studies on the pigments in roots and flowerheads of field-grown *G. verum* revealed a similar incapacity of the previously used procedures to deliver pure compounds and we can only conclude that much of the previous work on *G. verum* has involved the use of seriously impure samples from which the main (isomeric?) component has been characterized. This may account for several minor discrepancies in NMR spectra and MS fragmentation patterns between our samples and the values recorded for apparently authentic standards.

Analytical-HPLC revealed 46 red or yellow pigments in the pooled extract (corresponding to 0.05–1% of the dry weight in the individual cultures) of which the main eight were highly purified and characterized. All eight

*Author to whom correspondence should be addressed.

† Present address: Medical Unit, London Hospital Medical College, London E1 1BB.

were readily identified by characteristic loss of CO under EIMS to be anthraquinones and structures were assigned on the basis of spectral data previously collated for the class [3–5]. In particular: (i) *peri*-OH groups were revealed by a shift (δ180→186) in the ^{13}C-carbonyl signal and by an O-H resonance (*ca* δ13.2) in the ^{1}H NMR spectrum, both due to hydrogen bonding; (ii) similar chelation was revealed by a shift (1670 → 1625 cm^{-1}) in the characteristic carbonyl stretching frequency and an O-H band (at *ca* 3180 cm^{-1}) in the IR; (iii) in the absence of *peri*-OH groups red–violet colours were induced on basification of ethanolic solutions of the compounds; (iv) UV-VIS band maxima at *ca* 485 and 576 nm, respectively, corresponded to 1,3- and 1,2-dihydroxylation; (v) significant fragmentation in the EIMS to give ions $[M-H_2O]^+$ and $[M-H_2O-CHO]^+$ indicated a *peri*-OMe group [17]; (vi) two multiplets (each 2H) at *ca* δ7.75 and 8.24 in the ^{1}H NMR spectrum indicated an unsubstituted A ring [cf. **1**] (vii) C_1-Me singlets occur at δ2.60–3.00, whereas C_2-Me signals are at δ2.20; and (viii) C_1-OH signals are readily detectable when $CDCl_3$ is used as solvent, whereas C_2-OH signals often cannot be detected (unchelated H rapidly exchanges with traces of water in the solvent); however, the latter signals are apparent in C_6D_6 as solvent.

On the basis of such correlations the main pigment (48% of total purified) was characterized as 1,3-dihydroxy-2-methoxymethylanthraquinone (**2**; lucidin-ω-methyl ether): all spectral data were in excellent agreement with those reported for the compound extracted from roots of other Rubiaceae [18, 19] and from cell cultures of *Morinda citrifolia* [20]. This compound may be an artifact of the extraction method as others have found this methyl ether when the standard extraction solvent methanol is used [3–5]. Nevertheless, our analysis demonstrates the ready occurrence of the lucidin skeleton in our culture lines. Other compounds purified from the culture extracts that have not been reported as constituents of field-grown *G. verum* are: **3–6** and **7**. Compound **3** (1,3-dimethoxy-2-hydroxyanthraquinone) was assigned on the basis of the ^{1}H NMR spectrum and the close similarity of the MS-fragmentation pattern with that of 1-methoxy-2-hydroxyanthraquinone (alizarin-1-methyl ether; **9** [5]) in contrast with the completely different spectral (UV-VIS; MS; NMR) properties of synthetic and natural samples of the 1,4-dimethoxy-2-hydroxy isomer [21–23]. Compound **4** (1,3-dihydroxy-2-acetoxyanthraquinone) as assigned had spectral properties very similar to the anthraquinone of the same structure isolated from roots of other Rubiaceae [3, 24] and Verbenaceae [3] species. Compound **5** (1-hydroxy-2-hydroxymethyl anthraquinone) exhibited a ^{1}H NMR spectrum (best resolved with C_6D_6 as solvent) indicative of an unsubstituted A ring and a disubstituted C ring with a *peri*-OH group. Spectra (NMR; MS; UV-VIS) agreed with those for a compound of the same structure synthesized [18] and also occurring in foliage of *Digitalis* spp. [25, 26], and in roots and cell cultures of a number of the Rubiaceae [3, 5]. Compound **6** (1,3-dihydroxy-2-hydroxymethyl-6 methoxyanthraquinone or its 7-methoxy isomer) had MS and NMR (including solvent shifts) spectra markedly similar to **2** and the UV-VIS spectrum suggested 1,3-dihydroxylation. Analysis of the ^{1}H NMR signals indicated that the C ring contained MeO at either C-6 or C-7, but no decision could be made because of high symmetry of the A-B portion of the molecule. Comparison of the spectral data with those for similarly substituted anthraquinones prepared synthetically [3] suggest our compound to be the 6-isomer, but a specific synthesis is necessary to confirm this. Compound **7** (1,6-dihydroxy-2-methylanthraquinone or its 7-hydroxy isomer) was assigned similarly and again no firm conclusion could be reached. The UV-VIS and NMR spectra of **7** were similar to those of the 6-isomer obtained synthetically [27] and claimed to be isolated from callus of a *Cinchona* spp. [28]. The anthraquinones of *Galium* spp. are produced by the shikimate–glutarate–mevalonate route [10] and only five previous examples (none in *G. verum*) are known in which substitution in ring A occurs [29–31].

Two anthraquinones were also obtained that have previously been found in field-grown specimens of *G. verum* [32]. Compounds **8** (1,3-dihydroxy-2-methylanthraquinone) and **9** (1-methoxy-2-hydroxyanthraquinone) were characterized spectroscopically and their identities confirmed by comparison with published spectra [3–5, 28]. The anthraquinones **10–12** which are major components of field-grown *G. verum* were not major constituents of the cell cultures. A solid was isolated in appreciable yield (5% total) from the cultures and was characterized as a *trans*-caffeic acid methyl ester. This gave a UV-VIS and ^{1}H NMR spectra very similar to **13** which could be isolated from flowerheads of the plant [White, J., unpublished results], but a positive identification between the possible isomers could not be made with available NMR techniques in the absence of standards prepared synthetically.

EXPERIMENTAL

Culture methods. Specimens of *G. verum* were collected in water-meadows at East Hyde, Bedfordshire. The species readily hybridizes with other *Galium* species [33] and our samples were identified by Dr P. Yeo (Cambridge Botanic Garden) and specimens deposited in the UCL Botanic Garden. Explants of stem (50 × 5 mm) were established on M & S basal medium (ex. Flow Labs., Irvine, Ayrshire) supplemented with NAA (0.5 g l^{-1}), kinetin (0.2 g l^{-1}) and sucrose (3% wt/v) that was adjusted to pH 5.5 before autoclaving and solidification with agar (Oxoid, No.3; 1% wt/v). Callus was readily established (*ca* 80% success) within 4 weeks and was maintained for 9 sub-cultures of 21 day cycles at 24° under continuous illumination (600 lux; Phillips 'warm white', λ_{max} 580 nm) before different regimes were tested.

Extraction and purification. Pooled callus (161 g) was ground with acid-washed sand (8 g) under liquid N_2 with redistilled MeOH (8 l) and the residue repeatedly washed with MeOH to remove all colour. After removal of solvent, the extract was washed (2 × 500 ml; hexane);

concd and the glycosides present cleaved with dilute HCl [34]. In preliminary screening of the pigmentation of the different callus lines, the absorbance at λ 410 nm was measured and the anthraquinone content established in terms of equivalents of alizarin (λ_{max} 434 nm; ε 5.5×10^3; 80% EtOH).

HPLC sepns were carried out using a Gilson chromatograph controlled by Gilson 714 software run on an IBM PS/2-30 computer connected to either a Bischoff 3110-refractive index detector or an LKB-2140 UV photodiode array detector operating at 190–370 nm (256 data points). Data collected by the UV detector were further analysed using LKB Wavescan software so as to present data in 3D on a (λ, t, A) or a contour plot. Samples were run on analytical mode (1 ml min^{-1}, 1 μl) or prep. mode (4.9 ml min^{-1}; 20–30 injections; 200 μl) with ultrafiltration (0.2 μm Millipore) before injection. Normal phase (NP)-HPLC was carried out using silica gel (Nucleosil-100, 5 μm) on 250×4.6 mm or 250×10 mm columns for analyt. or prep. scale, respectively, with a guard column (50×4.6 mm) in each case. Reverse phase (RP)-HPLC utilized Spherisorb ODS2 (5 μm) on columns of the same dimensions. For (RP)-HPLC solvent systems were SI (A = H_2O, B = MeOH); S2 (A = H_2O, B = 95% MeCN + 5% H_2O) and for (NP)-HPLC: S3 (A = hexane, B = hexane 75% + 25% *iso*-PrOH) and S4 (A = hexane, B = EtOH). TFA (0.1%) was added to all systems to eliminate tailing.

The systematic purification of **2**–**9** and **13** involved the following network of chromatographic steps (the figs in brackets signify the proportions of the component **B** in the binary mixt.): thus, **9** was isolated by the steps S1 (71), S2 (35), S3 (20), S1 (70); **3** by these 4 steps followed by repeated S1 (70); **6** by S1 (71), S2 (35), S2 (60), S2 (50), S2 (45) and S1 (70); **8** by S1 (71), 2 ×; S2 (60), S1 (70), 2 ×; S4 (10); **7** by the same sequence; **4** by the first 5 steps of the sequence (as for **8**); **13** by S1 (71), 2 ×; S2 (35), S1 (44); **2** by S1 (71), 2 ×; S1 (70); and **5** by S1 (71), 2 ×; and S2 (45), 2 ×.

All compounds were estimated to be > 98% homogeneous, at min. by analyt.-HPLC on at least 3 systems using both detectors in peak-profiling mode. These assignments were confirmed by ion-profiling of the major ions in the EIMS at intervals (6 sec) up to 10 after introduction of the sample.

Structural assignments. NMR spectra were measured using either a Varian VXR-400 (^{1}H at 400 MHz; ^{13}C at 100.1 MHz) or JEOL GSX-FT500 (^{1}H at 500 MHz) spectrometer in $CDCl_3$ or C_6D_6 and the chemical shifts are given in δ values (ppm) with TMS as the int. standard. ^{13}C spectra were assigned with broadband decoupling of the protons and by the *J*-modulated spin echo technique (APT). Broadband decoupled spectra to yield quantitative ^{13}C spectra (e.g. signal area proportional to no. of carbons) were achieved by either inverse-gated decoupling or by the use of long pulse delays. In several cases second-order spectra in the aromatic region were elucidated using a spin-simulation programme (ex. Varian Associates. Palo Alto, CA, U.S.A.) with appropriate estimated chemical shifts and coupling constants to reproduce the observed patterns with high fidelity.

EIMS: (using a VG7070 instrument) was carried out at 70 eV with use of the NBS database; CIMS used NH_3 as carrier gas and FAB-MS utilized thioglycerol or *m*-nitrobenzoic acid as liquid matrices.

FT-IR: Nicolet 205 spectrometer 400–4000 cm^{-1} with microcell (0.1 mm); UV-VIS: Perkin-Elmer λ-16 with cell (1 cm).

Compound **2**. Red solid (19.3 mg; 49% of purified products). CIMS: *m/z* (rel. int.) 285 $[M + H]^+$ (100), 270 $[M + NH_3 - MeOH]^+$ (45). EIMS: *m/z* (rel. int.) 284.0692 $[M]^+$ ($C_{16}H_{12}O_5$ requires 284.0685), (7), 269 $[M - Me]^+$ (5), 255 $[M - CHO]^+$ (4), 254 $[M - CH_2O]^+$ (27), 253 $[M - OMe]^+$ (22), 252 $[M - MeOH]^+$ (100), 224 $[252 - CO]^+$ (7), 196 $[224 - CO]^+$ (21), 168 $[196 - CO]^+$ (9), 139 $[168 - CHO]^+$ (13). ^{13}C NMR: (100.1 MHz, $CDCl_3$): δ 186.9, 182.2, 164.0, 161.8, 134.1, 134.1, 134.0, 133.5, 133.5, 127.3, 126.7, 114.3, 109.8, 109.6, 68.9, 59.4. ^{1}H NMR (400 MHz, $CDCl_3$): δ 13.27 (1H, *s*, disappeared on shaking with D_2O), 9.37 (1H, *s*, disappeared on shaking with D_2O), 8.22–8.28 (2H, *m*), 7.72–7.80 (2H, *m*), 7.28 (1H, *s*), 4.91 (2H, *s*), 3.55 (3H, *s*); (C_6D_6): δ 13.71 (1H, *s*, disappeared on shaking with D_2O), 9.37 (1H, *s*, disappeared on shaking with D_2O), 8.21–8.22 (2H, *m*), 7.68 (1H, *s*), 6.95–7.01 (2H, *m*), 4.50 (2H, *s*), 2.70 (3H, *s*). IR ν_{max}^{KBr} cm^{-1}: 3180 (OH), 1670 (C = O), 1625 (C = O, chelated), 1595. UV-VIS λ_{max}^{EtOH} nm: 242 (sh), 246, 282, 334, 415; $\lambda_{max}^{EtOH-OH^-}$ nm: 249, 268, 299 (sh), 313, 330 (sh), 502.

Compound **3**. Yellow solid (0.7 mg; 1.8%). CIMS: *m/z* (rel. int.) 285 $[M + H]^+$ (100). EIMS: *m/z* (rel. int.) 284.0679 $[M]^+$ ($C_{16}H_{12}O_5$ requires 284.0685), (77), 269 $[M - Me]^+$ (17), 267 $[M - OH]^+$ (26), 266 $[M - H_2O]^+$ (100), 265 (57), 255 (14), 254 (19), 249 (17), 241 (53), 238 (23), 237 (73), 236 (26), 223 (16), 220 (11), 211 (21), 208 (24), 183 (18), 181 (13), 170 (58), 155 (11), 142 (15), 139 (14), 126 (23), 114 (32), 114 (32), 113 (29). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.20–8.27 (2H, *m*), 7.71–7.78 (2H, *m*), 7.70 (1H, *s*), 4.08 (3H, *s*), 3.99 (3H, *s*); (CD_3OD): δ 8.15–8.23 (2H, *m*), 7.75–7.83 (2H, *m*), 7.70 (1H, *s*), 4.05 (3H, *s*), 3.91 (3H, *s*); (C_6D_6): δ 8.24–8.27 (2H, *m*), 7.61 (1H, *s*), 7.12 (2H, *m*), 5.80 (1H, s, disappeared on shaking with D_2O), 3.75 (3H, *s*), 3.00 (3H, *s*). UV-VIS λ_{max}^{EtOH} nm: 226, 282, 318; $\lambda_{max}^{EtOH-OH^-}$ nm: 230, 260, 319, 519.

Compound **4**. Yellow solid. (3.2 mg; 8%). CIMS: *m/z* (rel. int.) 299 $[M + H]^+$ (100), 284 $[M + NH_4 - MeOH]^+$ (12). EIMS: *m/z* (rel. int.) 298.0489 $[M]^+$ ($C_{16}O_{10}O_6$ requires 298.0477), (21), 266 $[M - MeOH]^+$ (100), 238 $[266 - CO]^+$ (33), 210 $[238 - CO]^+$ (5), 182 $[210 - CO]^+$ (7), 154 $[182 - CO]^+$ (7), 126 $[154 - CO]^+$ (20). ^{1}H NMR (400 MHz, $CDCl_3$): δ 14.81 (1H, sharp *s*, disappeared on shaking with D_2O), 12.64 (1H, sharp *s*, disappeared on shaking with D_2O), 8.30–8.33 (1H, *m*), 8.24–8.27 (1H, *m*), 7.76–7.84 (2H, *m*), 7.38 (1H, *s*), 4.06 (3H, *s*). UV-VIS λ_{max}^{EtOH} nm: 246, 285, 335, 414; $\lambda_{max}^{EtOH-OH^-}$ nm: 221, 273, 312 (sh), 350 (sh), 510.

Compound **5**. Yellow solid (2.0 mg; 5%). CIMS: *m/z* (rel. int.) 255 $[M + H]^+$. EIMS: *m/z* (rel. int.) 254.0582 $[M]^+$ ($C_{15}H_{10}O_4$ requires 254.0579), (89), 236 $[M - H_2O]^+$ (17), 226 $[254 - CO]^+$ (22), 225 $[254 - CHO]^+$ (100), 208 $[236 - CO]^+$ (19), 207 (80), 197 $[226 - CHO]^+$ (7),

180 [208 − CO]$^+$ (14), 152 [180 − CO]$^+$ (39), 151 [180 − CHO]$^+$ (22), 139 (14). ^{1}H NMR (500 MHz, $CDCl_3$): δ13.03 (1H, *s*, disappeared on shaking with D_2O), 8.28–8.32 (2H, *m*), 7.76 (2H, *m*), 7.85 (1H, *d*, J = 8 Hz), 7.82 (2H, *m*), 7.76 (1H, *d*, J = 8 Hz), 5.40 (1H, (*br*) *s*, disappeared on shaking with D_2O), 4.85 (2H, *s*); (C_6D_6); δ13.19 (1H, *s*), 8.16–8.19 (1H, *m*), 8.03–8.06 (1H, *m*), 7.83 (1H, *d*, J = 7.8 Hz), 7.36 (1H, *d*, J = 7.8 Hz), 6.99–7.03 (2H, *m*), 4.54 (2H, *s*). UV-VIS λ_{max}^{EtOH} nm: 224, 245 (sh), 254, 280 (sh), 326, 407; $\lambda_{max}^{EtOH-OH^-}$ nm: 222, 250, 272 (sh), 311, 504.

Compound **6**. Yellow solid (1.6 mg; 4%). CIMS: *m/z* (rel. int.) 315 [M + H]$^+$ (37), 300 [M + NH_3 − MeOH]$^+$ (42). EIMS: *m/z* (rel. int.) 314 [M]$^+$ (7), 299 [M − Me]$^+$ (6), 285 [M − CHO]$^+$ (9), 284 [M − CH_2O]$^+$ (67), 283 [M − OMe]$^+$ (25), 282 [M − MeOH]$^+$ (100), 256 [284 − CO]$^+$ (3), 255 [283 − CO]$^+$ (5), 254 [282 − CO]$^+$ (6), 226 [254 − CO]$^+$ (8), 198 [226 − CO]$^+$ (2). ^{1}H NMR (500 MHz, $CDCl_3$): δ13.39 (1H, *s*, disappeared on shaking with D_2O), 9.30 (1H, (*br*) *s*, disappeared on shaking with D_2O), 8.20 (1H, *d* J = 8.6 Hz), 7.68 (1H, *d*, J = 2.7 Hz), 7.28 (1H, *s*), 7.23 (1H, *dd*, J = 2.7, 8.6 Hz), 4.91 (2H, *s*), 3.96 (3H, *s*), 3.55 (3H, *s*); (CD_3OD): δ8.15 (1H, *d*, J = 8.3 Hz), 7.59 (1H, *d*, J = 2.6 Hz), 7.28 (1H, *dd*, J = 2.6, 8.3 Hz), 7.18 (1H, *s*); (C_6D_6): δ13.96 (1H, *s*, disappeared on shaking with D_2O), 9.34 (1H, *s*, disappeared on shaking with D_2O), 8.14 (1H, *d*, J = 8.6 Hz), 7.75 (1H, *s*), 7.67 (1H, *d*, J = 2.7 Hz), 6.77 (1H, *dd*, J = 2.7, 8.6 Hz), 4.52 (2H, *s*), 3.10 (3H, *s*), 2.69 (3H, *s*). UV-VIS λ_{max}^{EtOH} nm: 275, 280 (sh), 308 (sh), 340, 430; $\lambda_{max}^{EtOH-OH^-}$ nm: 262, 303 (sh), 327, 505.

Compound **7**. Yellow solid (1.2 mg; 3%) CIMS: *m/z* (rel. int.) 255 [M + H]$^+$ EIMS: *m/z* (rel. int.) 254.0569 [M]$^+$ ($C_{15}H_{10}O_5$ requires 254.0579), (100), 253 (5), 252 (5), 237 (1), 226 (5), 225 (7), 198 (2), 197 (10), 181 (2), 169 (3), 152 (2), 151 (1), 149 (1), 147 (1), 141 (3), 139 (3), 127 (3), 121 (3). ^{1}H NMR (400 MHz, $CDCl_3$): δ13.06 (1H, *s*, disappeared on shaking with D_2O), 8.24 (1H, *d*, J = 8.4 Hz), 7.71 (1H, *d*, J = 7.6 Hz), 7.63 (1H, *d*, J = 2.7 Hz), 7.49 (1H, (*br*) *d*, 247, 270, 283 (sh), 294 (sh), 388 (sh), 412, 433 (sh); $\lambda_{max}^{EtOH-OH^-}$ nm: 216, 310, 345 (sh), 501.

Compound **8**. Yellow solid (3.9 mg; 10%). CIMS: *m/z* (rel. int.) 255 [M + H]$^+$. EIMS: *m/z* (rel. int.) 254.0483 [M]$^+$ ($C_{15}H_{10}O_4$ requires 254.0479), (100), 236 [M − H_2O]$^+$ (5), 226 [M − CO]$^+$ (10), 225 [M − CHO]$^+$ (10), 208 [236 − CO]$^+$ (4), 197 [225 − CO]$^+$ (9), 180 [208 − CO]$^+$ (5), 169 [197 − CO]$^+$ (4), 152 (10), 141 (13), 115 (15), 105 (21). ^{1}H NMR (400 MHz, $CDCl_3$): δ13.19 (1H, *s*, disappeared on shaking with D_2O), 8.22–8.30 (2H, *m*), 7.74–7.80 (2H, *m*), 7.27 (1H, *s*), 2.23 (3H, *s*). UV-VIS λ_{max}^{EtOH} nm: 242 (sh), 279, 310, 414; $\lambda_{max}^{EtOH-OH^-}$ nm 225, 300 (sh), 312, 495.

Compound **9**. Orange solid (4.9 mg; 12.3%). CIMS: *m/z* (rel. int.) 255 [M + H]$^+$. EIMS: *m/z* (rel. int.) 254.0584 [M]$^+$ ($C_{15}H_{10}O_4$ requires 254.0579), (40), 237 [M − OH]$^+$ (8), 236 [M − H_2O]$^+$ (37), 225 [M − CHO]$^+$ (13), 211 [M − COMe]$^+$ (12), 209 [M − OH − CO]$^+$ (17), 208 [M − H_2O − CO]$^+$ (100), 183 [M − COMe − CO]$^+$ (15), 180 [M − H_2O − 2CO]$^+$ (10), 168 (13), 155 [M − COMe − 2CO]$^+$ (9), 152 (19), 139 (25), 127 [155 − CO]$^+$ (23). ^{1}H NMR: (400 MHz, $CDCl_3$): δ8.22–8.28 (2H, *m*), 8.13 (1H, *d*, J = 8.5 Hz), 7.72–7.78 (2H, *m*), 7.34 (1H, *d*, J = 8.5 Hz), 6.65 (1H, *br d s*, disappeared on shaking with D_2O), 4.02 (3H, *s*). UV-VIS λ_{max}^{EtOH} nm: 247, 270, 284 (sh), 330, 381; $\lambda_{max}^{EtOH-OH^-}$ nm 249, 268 (sh), 315, 498.

Compound **13**. Solid (2.1 mg; 5%). CIMS: *m/z* (rel. int.) 209 [M + H]$^+$. EIMS: *m/z* (rel. int.) 208.0723 [M]$^+$ ($C_{11}H_{12}O_4$ requires 208.0736), (100), 193 [M − Me]$^+$ (3), 177 [M − OMe]$^+$ (69), 149 (12), 145 (27), 134 (11), 133 (14), 117 (13). ^{1}H NMR (400 MHz, $CDCl_3$): δ7.60 (1H, *d*, J = 15.9 Hz), 7.06 (1H, *dd*, J = 8.2, 1.9 Hz), 7.01 (1H, *d*, J = 1.9 Hz), 6.90 (1H, *d*, J = 8.2 Hz), 6.27 (1H, *d*, J = 15.9 Hz), 3.91 (3H, *s*), 3.78 (3H, *s*), UV-VIS λ_{max}^{EtOH} nm: 234, 299 (sh), 324. $\lambda_{max}^{EtOH-OH^-}$ nm: 250, 300, 309, 379.

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