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BIOSYNTHESIS OF ANTHRAQUINONES AND RELATED COMPOUNDS IN *GALIUM MOLLUGO* CELL SUSPENSION CULTURES*

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Key Word Index—*Galium mollugo*; Rubiaceae; lucidin-3-primeveroside; 1,4-dihydroxy-3-prenyl-2-naphthoic acid methyl ester diglucoside; biosynthesis; cell suspension culture; ^{13}C NMR; mass spectrometry.

Abstract—From *Galium mollugo* cell suspension cultures, 1,4-dihydroxy-3-prenyl-2-naphtholic acid methyl ester diglucoside was isolated along with anthraquinones and mollugin. Production of the diglucoside was much increased by administering 2-succinylbenzoate to the cultures. The incorporation of 2-succinylbenzoate into lucidin-3-primeveroside, mollugin and the diglucoside in the mode so far proposed for rubiaceous anthraquinones was verified by administration of ^{13}C-labelled 2-succinylbenzoate to the cell cultures.

INTRODUCTION

Anthraquinones are found in many Rubiaceae [1]. Unlike acetate–malonate derived microbial or polygonaceous anthraquinones such as emodin, the rubiaceous anthraquinones are biosynthesized from mevalonate and 4-(2′-carboxyphenyl)-4-oxo-butanoic acid (2-succinylbenzoic acid, OSB) (**1**), which is derived from shikimate and 2-oxoglutarate [2–6]. From the results of the degradation of labelled anthraquinones alizarin (**2**) and morindone (**3**), isolated after administration of [^{14}C]shikimate to *Rubia tinctorum* or *Morinda citrifolia*, it was demonstrated that the carboxy group of shikimate gives rise to C-9 of these compounds [5, 6]. Furthermore, through administration of [5-^{14}C]- and [2-^{14}C]MVA to the two plants, the incorporation of C-5 of MVA into C-4 of alizarin (**2**) and of C-2 of MVA into C-11 of pseudopurpurin (**4**) were demonstrated [4–6].‖

It was thus deduced that these anthraquinones are biosynthesized via prenylation at the position corresponding to C-3 of **1**. This prenylation mode is different from that in the biosynthesis of microbial menaquinone (**5**) [9] as well as of catalponol (**6**) and catalpalactone (**7**) in *Catalpa ovata* [10], all of which are formed via prenylation at the position corresponding to C-2 of **1**.

We thus re-examined the prenylation mode in the biosynthesis of these anthraquinones through administration of ^{13}C-labelled **1** to *Galium mollugo* L. cell cultures, which are known to produce several anthraquinones and mollugin (**8**) and served as the material for biosynthetic experiments of anthraquinones [11, 12].

RESULTS AND DISCUSSION

On re-examination of the constituents of the cell cultures, a trace of a new substance, 2-carbomethoxy-3-prenyl-1,4-naphthohydroquinone di-β-glucoside (**9**), was isolated, along with the known constituents lucidin-3-primeveroside (**10**), purpurin (**11**) and mollugin (**8**).¶ It was found that the production of the new compound was much increased by administration of **1** to the cell cultures.

Substance **9**, $C_{29}H_{18}O_{14} \cdot 1/2\ H_2O$, was obtained as colourless needles with the following spectral data: UV λ_{max}^{MeOH} nm: 231, 285, 295 and 330 (log ε 4.57, 3.79, 3.72 and 3.29); IR ν_{max}^{KBr} cm^{-1}: 3360, 1710, 1700 and 1590; ^1H NMR (DMSO-d_6): δ 1.63 and 1.68 (each *s*, =C–Me_2), 2.99–3.92 (C_2–C_6 protons of sugars), 3.74 (*d* (*br*), *J* = 6.5 Hz, –CH_2–CH=), 3.78 (*s*, –COOMe), 4.58 and 4.73 (each *d*, *J* = 7.5 Hz, 2 anomeric protons), 5.12 (*t* (*br*), *J* = 6.5 Hz –CH_2CH=C<), 7.50–7.63 and 8.56–8.63 (A_2B_2 type, 4 aromatic protons). From these data this substance was assumed to be 2-carbomethoxy-3-prenyl-1,4-naphthohydroquinone diglucoside. The ^{13}C NMR spectral data (Table 1) of **9** also supported this structure. Especially, the signals of the sugar moieties, each observed as a double line, were in accord with those of β-glucose [13], suggesting a di-β-glucoside structure. Hydrolysis of **9** with 5% hydrochloric acid gave dihydromollugin (**12**) [14] as an aglucone-derived substance as well as glucose as the sugar component. Although **9** was not hydrolysed by β-glucosidase contained in emulsin, it was hydrolysed with β-glucosidase prepared from Takadiastase [15] to give the following three products: 2-carbomethoxy-3-prenyl-1,4-naphthoquinone (**13**) [16, 17], an oxidation product of the aglucone as the main product, and

*Part 19 in the series "Quinones and Related Compounds in Higher Plants". Part of this study was reported as a preliminary communication in (1977) *J. Chem. Soc. Chem. Commun.* 957. For Part 18 see Inoue, K., Ueda, S., Nayeshiro, H. and Inouye, H. (1983) *Phytochemistry* **22**, 737.

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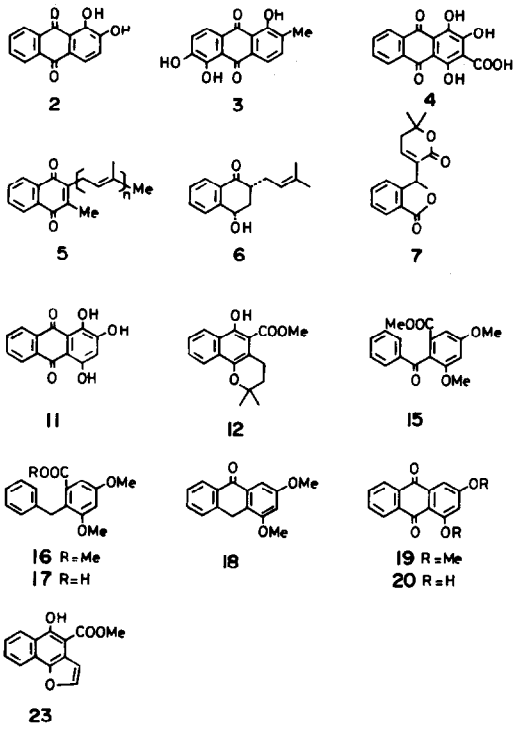
‖On somewhat prolonged administration of [2-^{14}C]MVA to *R. tinctorum*, incorporation of the label into C-1 and C-11 of anthraquinones was also observed [7, 8].

¶Other anthraquinones detected concurrently on TLC have not been further examined.



mollugin (**8**), a cyclic ether, as a minor product. Thus the structure of **9** was elucidated as 2-carbomethoxy-3-prenyl-1,4-naphthohydroquinone di-β-glucoside.

4-(2'-[2'-*Carboxy*-^{13}C]carboxyphenyl)-4-oxobutanoic acid (**1**) prepared in the usual way [18] was then administered to the *G. mollugo* cell cultures whose quinone content had been reduced through steady-state continuous culturing in a chemostat under phosphate limiting conditions [19]. After 8 days under the usual incubation conditions, the cultured cells were lyophilized and extracted with ethanol. The ethanolic extract was then subjected to droplet countercurrent chromatography (DCCC) [20] to yield lucidin-3-primeveroside (**10**), mollugin (**8**) and diglucoside (**9**). The ^{13}C NMR spectra of **10** and its aglycone lucidin (**14**) showed that the respective lower-field carbonyl signals at δ 186.9 and 185.9 were remarkably enriched with ^{13}C. Thus it was concluded that ^{13}C was incorporated into the C-9 carbonyl group adjacent to the hydroxy group at C-1. This was also supported by the ^{1}H NMR spectra of **10** and **14**, i.e. whereas both ^{13}C-enriched substances obtained in this experiment exhibited a deformation of the B_2 part of the A_2B_2 system due to the C-5, C-6, C-7 and C-8 protons coupling with the adjacent ^{13}C, the C-4 proton signal of both substances did not suffer any deformation except for the slight broadening due to long-range coupling with ^{13}C. Therefore, the above-described deformation could be ascribed to the coupling with the ^{13}C-atom introduced into the 9-position.

Finally, the incorporation of a ^{13}C-atom into C-9 was unambiguously demonstrated by comparison of the spectrum of the isolated lucidin (**14**) with that of the synthetically prepared quinone specifically labelled at C-9 in the following way. [1-*Carbonyl*-^{13}C]benzoyl chloride and methyl 3,5-dimethoxybenzoate were subjected to Friedel–Crafts reaction to give benzophenone (**15**). Since it was foreseen that direct cyclization of **15** would lead to scrambling of the label between C-9 and C-10 of the product owing to the formation of a spiro intermediate, **15** was first reduced with Et_3SiH [21] to the corresponding diphenylmethane (**16**). The latter was hydrolysed to carboxydimethoxydiphenylmethane (**17**), followed by conversion to acid chloride and Friedel–Crafts reaction, to give 2,4-dimethoxyanthrone (**18**). This compound, after oxidation to 1,3-dimethoxyanthraquinone (**19**), was demethylated to 1,3-dihydroxyanthraquinone (**20**), which was finally formylated to lucidin (**14**) having the ^{13}C-label at C-9.

The enrichment factor of ^{13}C in lucidin-3-primeveroside (**10**) calculated on the basis of the mass spectrum of its hydrolysate lucidin (**14**) was found to amount to 72.2 atom % excess. As the enrichment factor of ^{13}C in the administered **1** was 86.0 atom % excess, the specific incorporation of **1** into anthraquinone **10** therefore amounted to 83.9%.

In the ^{13}C NMR spectrum of the ^{13}C-enriched diglucoside **9** isolated along with **8** and **10**, two signals assignable to phenolic ether-bearing carbons were observed at δ 147.0 and 147.8. Of these two signals, the latter was highly enriched. Furthermore, the signals of the corresponding carbons in **12**, the hydrolysis product of **9**, appeared at δ 141.6 and 156.2, of which the lower-field signal was highly enriched. The C-2 signal of **12** was observed as a doublet

Table 1. ^{13}C NMR spectra of 2-carbomethoxy-3-prenyl-1,4-naphthohydroquinone diglucoside (**9**)*, mollugin (**8**)† and dihydromollugin (**12**)

Carbon	**9** (CD_3OD)	**8** ($CDCl_3$)	**12** ($CDCl_3$)
1	148.2 *s*	156.5 *s*	156.2 *s*
2	127.7 *s*‡	102.3 *s*	105.3 *s*
3	128.5 *s*‡	112.6 *s*	111.6 *s*
4	147.9 *s*	141.6 *s*	141.6 *s*
5	124.3 *d*§	129.3 *d*‡	129.0 *d*‡
6	128.4 *d*	126.3 *d*‡	125.6 *d*‡
7	128.4 *d*	124.0 *d*‡	123.8 *d*‡
8	124.6 *d*§	121.9 *d*	121.6 *d*
9	130.9 *s*	125.1 *s*	124.4 *s*
10	133.0 *s*	129.0 *s*	129.5 *s*
11	27.5 *t*	128.8 *d*	33.3 *t*
12	127.2 *d*	122.3 *d*	23.3 *t*
13	130.5 *s*	74.7 *s*	73.0 *s*
14	18.4 *q*	26.9 *q*	26.5 *q*
15	25.8 *q*	26.9 *q*	26.5 *q*
16	171.0 *s*	172.5 *s*	173.0 *s*
17	53.0 *q*	52.2 *q*	52.0 *q*

*Signals of the glucose moieties are given in Experimental.

†The reported assignments of some signals of **8** [14] were corrected. Especially the signal at δ 141.6 was assigned to C-4, since it appeared as a singlet and the corresponding signal was also observed in the spectrum of **12**. The signal at δ 102.2 was assigned to C-2 because of the coupling with C-1 in the spectrum of the ^{13}C-enriched **8**.

‡, §Values with the same superscript in the same column are interchangeable.

at δ 105.6 ($J_{C_1-C_2}$ = 68.4 Hz) due to the coupling with ^{13}C. It was concluded that C-1 in **9** was enriched with ^{13}C. The enrichment factor of ^{13}C in **9** calculated on the basis of the mass spectrum of **12** was 80.3 atom % excess, i.e. the specific incorporation ratio of [^{13}C]-**1** into **9** amounted to 93.3%.

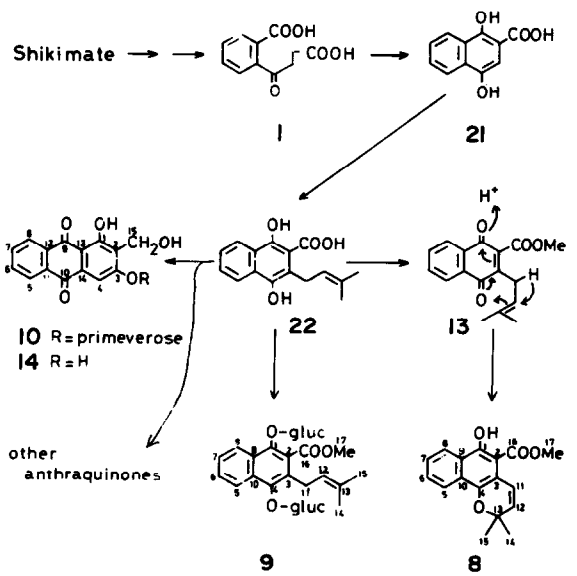
Furthermore, in the spectrum of mollugin (**8**), the signal at 156.5 due to C-1 was enriched. The enrichment factor of ^{13}C in **8** amounted to 68.1 atom % excess and the specific incorporation ratio, 79.1%.

The results described above clearly demonstrated that the series of compounds **8**, **9**, **10**, etc. are biosynthesized through a route involving, as key intermediates, 1,4-dihydroxynaphthoic acid (**21**) and 1,4-dihydroxy-3-prenyl-2-naphthoic acid (**22**) (Scheme 1). The observed specific incorporation ratios of **1** into the three metabolites **8**–**10** in the decreasing order of **9**, **10** and **8** can be explained by an unusual overflow production of the minor metabolite **9** triggered off through overproduction of **22**. This is caused by administration of **1** and cannot be regulated by the usual metabolism into anthraquinones and mollugin (**8**), etc.

As congeners of **9**, carbomethoxyprenyl-naphthoquinone (**13**) and furomollugin (**23**) occur in the original *G. mollugo* plant along with anthraquinones and mollugin (**8**) [16, 17, 22] so that **13** seems to be an important intermediate between **22** and **8** on the one hand, and between **22** and **23** on the other.

EXPERIMENTAL

General procedures. Mps uncorr; ^1H NMR (200 MHz) and ^{13}C NMR (50.1 MHz): TMS as the int. standard; MS: direct inlet at 70 eV; GLC: glass columns (2 m × 6 mm) packed with 3% OV-1 on 100–200 mesh Gas Chrom Q or with 1.5% OV-17 on 80–100 mesh Shimalite W, using N_2 as carrier gas (50 ml/min); TLC: silica gel GF_{254}; prep. TLC: PF_{254}. Spots were visualized under UV radiation. DCCC: descending method with $CHCl_3$–MeOH–H_2O (5:5:3) using Pyrex glass tubes (120 cm × 2.4 mm i.d.) connected by Teflon tubing (140 cm × 0.86 mm i.d.).



Scheme 1. Biosynthetic pathways for the formation of quinones in *G. mollugo* suspension cultures.

Cell culture and isolation of constituents from the cells. Suspension cultured cells of *G. mollugo* incubated in Gamborg B_5 medium supplemented with 2,4-D (1×10^{-5} M), NAA (2.7×10^{-6} M), IAA (3×10^{-6} M) and kinetin (1×10^{-6} M) (18 × 100 ml) for a period of 7 days were collected and lyophilized. The dried cells (22.7 g) were extracted with hot EtOH (1.4 l. × 3) and the EtOH soln was concd *in vacuo* to give a dark red residue (4.62 g), 2.0 g of which was triturated with the upper layer (20 ml) of a mixture of $CHCl_3$–MeOH–H_2O (5:5:3). The insoluble red ppt. was collected by filtration and recrystallized from pyridine–H_2O to give 5 mg purpurin (**11**) as red needles, mp 263°, which was identified with an authentic sample (mmp, IR and ^1H NMR). The filtrate obtained through the removal of the above described red ppt. was directly subjected to DCCC employing 51 distribution columns, and 8 ml fractions were collected. The residue (440 mg) obtained by concn of fractions 1–4 *in vacuo* was chromatographed on a silica gel column (10 g) with $CHCl_3$ as eluant. The first eluate (40 ml) was concd *in vacuo* to give a yellow crystalline residue (20 mg), which was purified through sublimation (130°/0.5 mmHg) to give mollugin (**8**) as yellow needles (15.6 mg), mp 125°. This substance was identified with an authentic sample (mmp, IR and ^1H NMR). A yellow residue (49 mg) obtained by concn of combined fractions 175–230 *in vacuo* was recrystallized from MeOH to give lucidin-3-primeveroside (**10**) as yellow needles (28 mg), mp 210–212°, $[\alpha]_D^{25}$ – 102° (*c* 1.0; DMF). This substance was identified with an authentic sample (mmp, IR and ^1H NMR). Combined fractions 310–390 were concd *in vacuo* to give a residue (50 mg), which was purified successively through charcoal CC (0.5 g) with MeOH as eluant and prep. TLC ($CHCl_3$–MeOH, 3:1). A band around R_f 0.20 was scraped off and eluted with $CHCl_3$–MeOH (1:1). The eluate was concd *in vacuo* to give 2-carbomethoxy-3-prenyl-1,4-naphthohydroquinone diglucoside (**9**) as colourless needles (2 mg).

Administration of 4-(2'-carboxyphenyl)-4-oxobutanoic acid (**1**) *to* G. mollugo *cell suspension culture and isolation of 2-carbomethoxy-3-prenyl-1,4-naphthohydroquinone diglucoside* (**9**). *G. mollugo* cell suspension cultures were shaken in the above-described medium (300 ml × 10) supplemented with **1** (700 mg × 10) for 7 days. The cells were collected, lyophilized and extracted with hot 80% EtOH. The aq. EtOH extract was concd *in vacuo* to give a dark red residue (84.6 g), which was suspended in H_2O (800 ml) and centrifuged to remove insoluble material mainly constituted of anthraquinones. The supernatant was shaken with C_6H_6 (500 ml × 2). The aq. layer was subsequently shaken with *n*-BuOH (1 l. × 5) and the combined *n*-BuOH layers were concd *in vacuo* to give an orange-yellow residue (10.3 g) which was dissolved in MeOH (400 ml). After removal of insoluble materials, the MeOH soln was poured onto a charcoal column and eluted with MeOH (1 l.). The combined eluates were concd *in vacuo* and the residue was recrystallized from MeOH–H_2O to give **9** as colourless needles (7.1 g), mp 240–242° (decomp.), $[\alpha]_D^{25}$ + 4.0° (*c* 0.5; MeOH). ^{13}C NMR of glucose moieties (CD_3OD): δ 63.2, 63.5 (each *t*, C-6',6''), 71.5, 71.8 (each *d*, C-4',4''), 75.5, 75.7 (each *d*, C-2',2''), 77.7, 77.9 (each *d*, C-3',3'',5',5''), 106.1, 106.3 (each *d*, C-1', 1''); (Found: C, 54.65; H, 6.36. $C_{29}H_{38}O_{14} \cdot 1/2\,H_2O$ requires: C, 54.63; H, 6.48%).

Acid hydrolysis of diglucoside (**9**). 5% HCl (5 ml) was added to a soln of **9** (50 mg) in MeOH (1 ml) and the whole was heated in a boiling water bath for 30 min. After being cooled, the reaction was diluted with H_2O (20 ml) and extracted with Et_2O (20 ml × 3). The Et_2O layer was washed with H_2O, dried and concd *in vacuo* to give a residue (24 mg), which was recrystallized from MeOH to give dihydromollugin (**12**) as light yellow needles

(20 mg), mp 99–100°. UV λ_{max}^{MeOH} nm (log ε): 262 (sh) (4.40), 270 (4.47), 324 (3.53) and 377 (3.52); IR ν_{max}^{KBr} cm^{-1}: 2950, 1640 and 1625; ^{1}H NMR ($CDCl_3$): δ 1.38 (6H, *s*, *gem.*-Me), 1.82 (2H, *t*, *J* = 7.0 Hz, ), 3.04 (2H, *t*, *J* = 7.0 Hz, ) 3.95 (3H, *s*, –COOMe), 7.27–7.70 (2H, *m*, H-6, 7), 8.02–8.40 (2H, *m*, H-5, 8), 12.08 (1H, *s*, chelated OH); Found: C, 71.06; H, 6.42. Calc. for $C_{17}H_{18}O_4$: C, 71.32; H, 6.34%. This substance was identical with dihydromollugin (**11**) obtained by the catalytic hydrogenation of mollugin (**8**) over Pd–C (mmp, IR and ^{1}H NMR). The H_2O-soluble part of the hydrolysate was concd *in vacuo*. The resulting residue, after trimethylsilylation, was found to be identical with pertrimethylsilylated glucose by GLC. Column temp. 190°, injection temp. 240°, OV-1: R_t 9 min (α-anomer), 13.5 min (β-anomer); OV-17: 5.2 min (α-anomer), 7.0 min (β-anomer).

Hydrolysis of diglucoside (**9**) *with a β-glucosidase fraction of Takadiastase.* A β-glucosidase fraction (5 ml) of Takadiastase was added to a suspension of **9** (50 mg) in deionized H_2O (5 ml) and the whole was shaken gently at 37° for 48 hr. After the addition of H_2O (10 ml), the reaction was extracted with Et_2O (20 ml × 3) and the Et_2O layer was washed with H_2O, dried and concd *in vacuo* to give a reddish yellow residue (23 mg), which was subjected to prep. TLC with petrol–$CHCl_3$ (3:2) as eluant. A yellow major band at R_f 0.32 was scraped off and extracted with $CHCl_3$ to give **13** (17 mg) as a yellow oil. UV λ_{max}^{MeOH} nm (log ε): 247 (4.24), 252 (4.24), 265 (sh) (4.10) and 334 (3.53); IR $\nu_{max}^{CHCl_3}$ cm^{-1}:1735, 1660, 1650 (sh), 1620 and 1590; ^{1}H NMR ($CDCl_3$): δ 1.71 (6H, *s* (*br*), *gem.*-Me), 3.23 (2H, *d*, *J* = 7.0 Hz, ), 3.91 (3H, *s*, –COOMe), 5.07 (1H, *t* (*br*), *J* = 7.0 Hz, ), 7.62–7.83 (2H, *m*, H-6, 7), 7.90–8.13 (2H, *m*, H-5, 8); ^{13}C NMR ($CDCl_3$): δ 17.9 (*q*, C-14), 25.7 (*q*, C-15), 27.2 (*t*, C-11), 52.5 (*q*, –COOMe), 118.1 (*d*, C-12), 126.0, 126.4 (each *d*, C-5,8), 131.0, 131.5 (each *s*, C-9,10), 133.7 (*d*, C-6,7), 135.2, 138.6 (each *s*, C-3 and C-13), 145.9 (*s*, C-2), 164.6 (*s*, –COOMe), 181.6, 183.8 (each *s*, C-4,1); MS *m/z* (rel. int.): 284 $[M]^+$ (39), 269 $[M-15]^+$ (23), 252 (44), 237 (base peak); (Found: 284.1028. Calc. for $C_{17}H_{16}O_4$: 284.1048). A minor band at R_f 0.52 was eluted with $CHCl_3$ and the residue of the eluate was sublimed *in vacuo* to give **8** as yellow plates (3 mg), mp 125°. This substance was identified with an authentic sample (mmp, IR and ^{1}H NMR).

4-(2′-[2′-Carboxy-^{13}C]*carboxyphenyl*)-4-*oxobutanoic acid* (**1**). Starting from methyl 4-(2′-bromophenyl)-4-oxobutanoate (3.0 g) and $Cu^{13}CN$ (enrichment factor 90% excess, 0.7 g), **1** (0.76 g) was prepared in the usual way [18]. Mp 136–137°, IR ν_{max}^{KBr} cm^{-1}: 2800, 1720, 1700; ^{13}C NMR (Me_2CO-d_6, measured at −60°)*: δ 174.4 (*s*, C-1), 168.5 (*s*, C-2′-COOH), 149.7 (*s*, C-1′); 135.7 (*d*, C-5′), 131.5 (*d*, C-4′), 127.3 (*s*, C-2′), 125.6 (*d*, C-3′), 123.6 (*d*, C-6′), 107.6 (*s*, C-4), 34.3 (*t*, C-3), 28.6 (*t*, C-2). High resolution EIMS, Found: 223.0563. $^{13}CC_{10}H_{10}O_5$ requires: 223.0562.

Administration of [2′-carboxy-^{13}C]-**1** *to* G. mollugo *cell suspension cultures. G. mollugo* cells in a continuous culture apparatus [19, 23] under steady-state phosphate limiting conditions were transferred to conical flasks (100 ml × 24) each containing [^{13}C]-**1** (15.5 mg) and incubated as ordinary shake cultures for 8 days. The cells were then collected and lyophilized. Dry cells (5.5 g) were extracted with hot EtOH (500 ml × 3) and the extract was concd *in vacuo* to give a reddish brown residue (0.97 g), which was subjected to DCCC, collecting 10 ml fractions. Fractions 1–5 obtained in the same way as described above gave mollugin (**8**) (5 mg), mp 125°. Fractions 106–150 gave lucidin-3-primeveroside (**10**) (12 mg), mp 210–212°, ^{13}C NMR (DMSO-d_6): δ 65.5, 67.9, 69.1, 69.3, 73.2, 76.3, 100.7, 103.9 (sugar carbons), 50.9 (*t*, C-15), 106.3 (*d*, C-4), 126.8 (*d*, C-5), 134.7 (each *d*, C-6, 7), 126.3 (*d*, C-8), 112.3 (*s*, C-11), 131.5 (*s*, C-14), 132.7 (*s*, C-13), 133.7 (*s*, C-12), 123.5 (*s*, C-2), 161.7 (*s*, C-3), 161.9 (*s*, C-1), 181.3 (*s*, C-10) and 186.9 (*s*, C-9); and fractions 201–340 afforded the diglucoside **9**, mp 240–242° (20 mg). ^{1}H NMR (DMSO-d_6): δ 8.52–8.67 (2H, deformed *m*, H-5,8). On hydrolysis with 5% HCl, primeveroside (**10**) gave lucidin (**14**), mp 300°, ^{13}C NMR (DMSO-d_6): δ 51.2 (*t*, C-15), 107.7 (*d*, C-4), 126.2 (*d*, C-8), 126.5 (*d*, C-5), 133.1 (*d*, C-7), 134.3 (*d*, C-6), 110.6 (*s*, C-11), 120.1 (*s*, C-2) 131.7 (*s*, C-14), 132.7 (*s*, C-13), 133.9 (*s*, C-12), 163.0 (*s*, C-3), 163.4 (*s*, C-1), 181.5 (*s*, C-10) and 185.9 (*s*, C-9); MS: *m/z* (rel. int.): 270 $[M]^+$ (2.2), 271 $[M+1]^+$ (6.2); and the diglucoside **9** gave dihydromollugin (**12**), mp 99–100°, MS: *m/z* (rel. int.): 286 $[M]^+$ (12.5), 287 $[M+1]^+$ (27.2).

Synthesis of [9-^{13}C]-*lucidin* (**14**). (a) 2-*Carbomethoxy*-4,6-*dimethoxy*[Carbonyl-^{13}C]*benzophenone* (**15**): To a soln of [*carboxy*-^{13}C]benzoyl chloride (enrichment factor 86% excess, 1.0 g) and methyl 3,5-dimethoxybenzoate (1.4 g) in dry $CHCl_2CHCl_2$ (10 ml) was added pulverized dry $AlCl_3$ (1.4 g) and the whole was stirred at room temp. for 24 hr. The reaction was poured into ice H_2O and extracted with $CHCl_3$ (200 ml × 3). The $CHCl_3$ layer was washed with H_2O, dried and concd to give a residue (1.5 g), which was recrystallized from MeOH to give [*carbonyl*-^{13}C]-**15** as colourless needles (0.6 g), mp 138–139°; UV λ_{max}^{MeOH} nm (log ε): 249 (4.08) and 310 (3.47); IR ν_{max}^{KBr} cm^{-1}: 1710, 1655 and 1590; ^{1}H NMR ($CDCl_3$): δ 3.62, 3.68 (each 3H, *s*, –OMe), 3.88 (3H, *s*, –COOMe), 6.68 (1H, *d*, *J* = 2.0 Hz, H-5), 7.16 (1H, *d*, *J* = 2.0 Hz, H-3), 7.33–7.55 (3H, *m*, H-3′,4′,5′), 7.67–7.87 (2H, deformed *m*, H-2′,6′); EIMS: *m/z* 301 $[M]^+$ ($^{13}CC_{16}H_{16}O_5$).

(b) 2-*Carbomethoxy*-4,6-*dimethoxy*[methylene-^{13}C]*diphenylmethane* (**16**): Et_3SiH (1.0 g) was added to a soln of [*carbonyl*-^{13}C]-**15** (600 mg) in CF_3COOH (340 mg) and the whole was stirred at room temp. for 48 hr. The reaction was concd *in vacuo* to give a residue, which was recrystallized from MeOH to give [*methylene*-^{13}C]-**16** as colourless plates (320 mg), mp 92–93°. ^{1}H NMR ($CDCl_3$): δ 3.72, 3.75 (each 3H, *s*, –OMe), 3.77 (3H, *s*, –COOMe), 4.27 (2H, *d*, $J_{^{13}C-H}$ = 128 Hz, Ar-$^{13}CH_2$–), 6.58 (1H, *d*, *J* = 2.0 Hz, H-5), 6.92 (1H, *d*, *J* = 2.0 Hz, H-3), 7.10 (5H, *s*, H-2′,3′,4′,5′,6′); EIMS: *m/z* 287 $[M]^+$ ($^{13}CC_{16}H_{18}O_4$).

(c) 2-*Carboxy*-4,6-*dimethoxy* [methylene-^{13}C] *diphenylmethane* (**17**): A soln of [*methylene*-^{13}C]-**16** (300 mg) in MeOH (3 ml) was added to 2 M aq. NaOH (19 ml) preheated to 80° and refluxed for 3 hr. The reaction was worked up in the usual manner and the product was recrystallized from HOAc to give [*methylene*-^{13}C]-**17** as colourless prisms (280 mg), mp 199–201°. ^{1}H NMR (Me_2CO-d_6): δ 3.80, 3.83 (each 3H, *s*, –OMe), 4.34 (2H, *d*, $J_{^{13}C-H}$ = 128 Hz, Ar-$^{13}CH_2$–), 6.73 (1H, *d*, *J* = 2.5 Hz, H-5), 6.33–7.00 (1H, *s* (*br*), –COOH), 7.03 (1H, *d*, *J* = 2.5 Hz, H-3), 7.11 (5H, *s*, H-2′, 3′, 4′, 5′, 6′). High resolution EIMS, Found: 273.1080. $^{13}CC_{15}H_{16}O_4$ requires: 273.1081.

(d) 2,4-*Dimethoxy*[10-^{13}C]*anthrone* (**18**): $SOCl_2$ (1.4 mg) was added to a soln of [*methylene*-^{13}C]-**17** (280 mg) in dry C_6H_6 (8 ml) and the whole was refluxed for 2 hr. After being cooled, the reaction was concd to give a residue (308 mg), which was dissolved *in situ* in dry $CHCl_2CHCl_2$ (1 ml) and cooled with ice. Pulverized dry $AlCl_3$ (200 mg) was added to the ice-cold soln with stirring and the stirring was continued for a further 10 min. The

*Compound **1** exists in soln as a mixture of keto and lactone (between carboxy at C-2′ and 4-keto groups) forms, the latter being predominant at low temperature. Besides the above carbon signals attributed to the lactol form, the spectrum showed the following small signals due to the minor keto form: δ 205.1 (*s*, C-4), 174.7 (*s*, C-1), 144.8 (*s*, C-1′), 133.5 (*d*, C-5′), 130.8 (*d*, C-3′ or C-4′), 130.5 (*d*, C-4′ or C-3′), 127.0 (*d*, C-6′), 128.7 (*s*, C-2′), 38.1 (*t*, C-3). Detailed data will be reported elsewhere.

reaction was further stirred at room temp. for 10 min, at 45° for 15 min and at 60° for 10 min. After cooling, the reaction mixture was poured into a mixture of 10% H_2SO_4 and ice and extracted with EtOAc (50 ml ×3). The EtOAc layer was washed successively with saturated aq. $NaHCO_3$ and H_2O, dried and concd to give a residue (270 mg), which was chromatographed on a silica gel column (30 g) with $CHCl_3$ as eluant, collecting 50 ml fractions. Combined fractions 7–10 were concd *in vacuo* to give a residue (123 mg), which was recrystallized from MeOH to yield [10-^{13}C]-**18** as light yellow needles (95 mg), mp 135–136°. UV λ_{max}^{MeOH} nm (log ε): 255 (4.16), 275 (sh) (4.08) and 343 (3.42); IR ν_{max}^{KBr} cm^{-1}: 1655 and 1590; ^{1}H NMR ($CDCl_3$): δ 3.86 (6H, *s*, –OMe × 2), 4.06 (2H, *d*, $J_{^{13}C-H}$ = 128 Hz, Ar-$^{13}CH_2$–), 6.64 (1H, *d*, *J* = 2.5 Hz, H-3), 7.38 (1H, *d*, *J* = 2.5 Hz, H-1), 7.45–7.55 (3H, *m*, H-5, 6, 7), 8.22–8.42 (1H, *m*, H-8); EIMS: *m/z* 255 [M]$^+$ ($^{13}CC_{15}H_{14}O_3$).

(e) 1,3-*Dimethoxy*[9-^{13}C]*anthraquinone* (**19**): An aq. soln (2 ml) of $Na_2Cr_2O_7 \cdot 2H_2O$ (106 mg) was added to a soln of [10-^{13}C]-**18** (90 mg) in HOAc (4 ml), prewarmed to 50–55°, with vigorous stirring. The reaction was gradually warmed to 80°, then kept at the same temp. for 10 min and then cooled to room temp. The resulting yellow ppt. was collected and recrystallized from MeOH to give [9-^{13}C] **19** as yellow needles (93 mg), mp 162–163.5°. ^{1}H NMR ($CDCl_3$): δ 3.90, 3.95 (each 3H, *s*, –OMe), 6.68 (1H, *d*, *J* = 2.0 Hz, H-2), 7.33 (1H, *d*, *J* = 2.0 Hz, H-4), 7.57–7.73 (2H, *m*, H-6, 7), 8.04–8.27 (2H, deformed *m*, H-5, 8); EIMS: *m/z* 269 [M]$^+$ ($^{13}CC_{15}H_{12}O_4$).

(f) 1,3-*Dihydroxy*[9-^{13}C]*anthraquinone* (**20**): A mixture of [9-^{13}C]-**19** (93 mg) and pyridinium chloride (1.6 g) was stirred at 160° to make a homogeneous soln, and then stirred at 180° for a further 6 hr. After cooling, H_2O (30 ml) was added to the solidified reaction mixture. The resulting ppt. was washed with H_2O and dissolved in 5% aq. Na_2CO_3 (30 ml). The insoluble material was filtered off and the filtrate was acidified with HCl. After washing with H_2O, the resulting ppt. was recrystallized from pyridine–H_2O to give [9-^{13}C]-**20** as yellowish brown needles (75 mg), mp 276–278°. ^{1}H NMR (DMSO-d_6): δ 6.50 (1H, *d*, *J* = 2.0 Hz, H-2), 7.03 (1H, *d*, *J* = 2.0 Hz, H-4), 7.63–8.17 (4H, deformed *m*, H-5, 6, 7, 8), 10.33–11.50 (1H, *s* (*br*), –OH), 12.58 (1H, *s*, chelated OH); EIMS: *m/z* 241 [M]$^+$ ($^{13}CC_{13}H_8O_4$).

(g) [9-^{13}C]*Lucidin* (**14**): A 3.5% aq. HCHO (0.5 ml) soln was added to a soln of [9-^{13}C]-**19** (75 mg) in 5% KOH (0.8 ml) and the whole was stirred at room temp. for 5 hr. The reaction was acidified with dilute HCl and the resulting ppt. was washed with H_2O and recrystallized from C_6H_6 to yield [9-^{13}C]-**14** as yellow needles (70 mg), mp 300°. UV λ_{max}^{MeOH} nm (log ε): 242 (4.34), 246 (4.35), 280 (4.35), 332 (3.40) and 410 (3.75); IR ν_{max}^{KBr} cm^{-1}: 3380, 1655, 1610 and 1585; ^{1}H NMR (DMSO-d_6): δ 4.50 (2H, *s*, Ar–CH_2–), 7.16 (1H, *s*, H-4), 7.72–8.23 (4H, deformed *m*, H-5, 6, 7, 8), 13.07 (1H, *s*, chelated OH). ^{13}C NMR (DMSO-d_6): the same as non-labelled **14** except for the strong signal at δ 185.9 (C-9). High resolution EIMS, Found: 271.0569. $^{13}C_1C_{14}H_{10}O_5$ requires: 271.0562.

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