Site of Prenylation in Anthraquinone Biosynthesis in Cell Cultures of *Galium mollugo*

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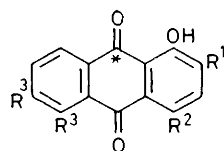
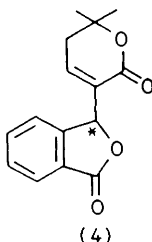
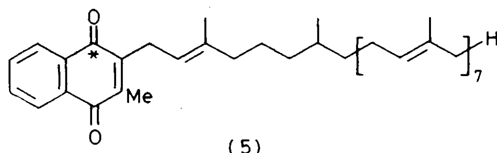
Summary The mode of prenylation during the biosynthesis of lucidin primeveroside was elucidated by locating the ^{13}C-label in lucidin primeveroside isolated from cell cultures of *Galium mollugo,* to which 4-(2′-[C^{13}]carboxyphenyl)-4-oxobutanoic acid was administered.

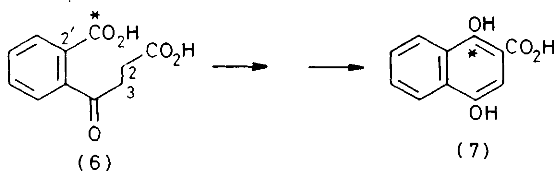
Rubiaceae are known to be a rich source of anthraquinones.[1] Recently, several anthraquinones[2–5] have also been isolated from cell cultures of the plants *Morinda citrifolia* L. and *Galium mollugo* L. belonging to this family. Feeding and degradation experiments with ^{14}C-labelled substances indicated that these anthraquinones, especially alizarin (**1**), purpurin (**2**), and morindone (**3**), are biosynthesized from mevalonic acid and 4-(2′-carboxyphenyl)-4-oxobutanoic acid (**6**). The intermediate (**6**) is derived from shikimic acid and 2-oxoglutaric acid, and prenylation during the biosynthesis of the anthraquinones is known to occur at the position corresponding to C-3 of (**6**).[6–9] On the other hand, naphthoquinone congeners such as catalpalactone (**4**)[10] and menaquinone [MK-9 (II-H_2)](**5**),[11] also derived from the same biosynthetic precursor (**6**), are known to be formed by prenylation at the position corresponding to C-2 of (**6**).

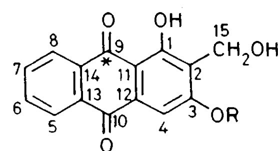


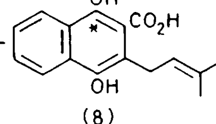
(1) R¹ = OH, R² = R³ = H
(2) R¹ = R² = OH, R³ = H
(3) R¹ = Me, R² = H, R³ = OH











(9) R = Primeverose
(10) R = H

Scheme

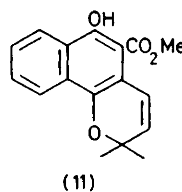
We therefore attempted to verify the mode of prenylation during the biosynthesis of anthraquinones in Rubiaceous plants with the aid of ^{13}C-labelling techniques using cell cultures of *G. mollugo*, which produce lucidin primeveroside (**9**) as the main product. 4-(2′-[^{13}C]Carboxyphenyl)-4-oxobutanoic acid (**6**), prepared according to the synthetic method used for the corresponding ^{14}C-labelled compound,[10] was administered to a cell suspension of *G. mollugo*, which had been cultured in a continuous culture system and was further incubated for six days. The ethanolic extract of the cultured cells (dry weight 11·66 g) was fractionated by droplet counter-current chromatography[12] yielding 25 mg of lucidin primeveroside (**9**), m.p. 210—212 °C, $[\alpha]_D^{20}$ $-102°$ (*c* 1·7, $HCONMe_2$) with an enrichment factor of 72·2 ^{13}C atom % excess (calculated from mass spectral data), besides some minor products. The glycoside (**9**) was hydrolysed with dil. HCl to give lucidin (**10**). ^{13}C N.m.r. data for (**9**) and (**10**) are in the Table. For both compounds

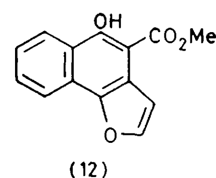
Table. ^{13}C N.m.r. data for lucidin primeveroside (**9**) and lucidin (**10**) (anthraquinone unit)

Carbon	(9)		(10)	
1	161·9[a]	(s)	163·4[a′]	(s)
2	123·5	(s)	120·1	(s)
3	161·7[a]	(s)	163·0[a′]	(s)
4	106·3	(d)	107·7	(d)
4	126·8[b]	(d)	126·5[b′]	(d)
6	134·7	(d)	134·3[c′]	(d)
7	134·7	(d)	133·1[c′]	(d)
8	126·3[b]	(d)	126·2[b′]	(d)
9	186·9	(s)	185·9	(s)
10	181·3	(s)	181·5	(s)
11	112·3	(s)	110·6	(s)
12	133·7[c]	(s)	133·9[d′]	(s)
13	132·7[c]	(s)	132·7[d′]	(s)
14	131·5[c]	(s)	131·7[d′]	(s)
15	50·9	(t)	51·2	(t)

[a–c], [a′–d′] Assignments for signals with the same letter are exchangeable.

signals assignable to the carbonyl carbons appear at δ *ca.* 186 and 181 p.p.m., and in both the cases the lower-field signals are strongly enhanced. These findings indicate that the ^{13}C-label of the precursor (**6**) was incorporated into the hydrogen-bonded C-9 carbonyl carbon of the anthraquinone. The ^{1}H n.m.r. spectrum (in CD_3SOCD_3) of lucidin primeveroside (**9**) also supports this conclusion.





The natural glycoside (**9**) shows in its ^{1}H n.m.r. spectrum typical A_2B_2 type signals at δ 7·81—7·98 and 8·06—8·26 for the protons at C-5, C-6, C-7, and C-8, while enriched (**9**) isolated from the above-mentioned cell culture shows a deformed pattern for the lower-field B_2 part of the signals which could be attributed to the long-range coupling of one of the protons with the vicinal ^{13}C. In contrast, the ^{13}C enriched glycoside (**9**) does not show any change in the shape of the signal (br. s, δ 7·46) for the C-4 proton. Based on these findings, the enriched carbon occupies position 9, but not 10. All these results prove the validity of the hitherto postulated biosynthetic pathway of anthraquinones in Rubiaceous plants *via* 1,4-dihydroxy-2-

naphthoic acid (**7**) and 1,4-dihydroxy-3-prenyl-2-naphthoic acid (**8**) (Scheme), which differs from the pathway for compounds (**4**) and (**5**) in the site of prenylation. It is likely that mollugin (**11**)[5,13] and furomollugin (**12**)[14] are also biosynthesized in plants from (**8**).

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