

# Multiple convergence in polyketide biosynthesis: a third folding mode to the anthraquinone chrysophanol

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## Experimental details

**Chemicals:** [1,2-<sup>13</sup>C<sub>2</sub>]NaOAc (99% isotopic enrichment) was purchased from Eurisotop (Saarbrücken, Germany).

**Biological material and sterile culture:** *Streptomyces* strain AK 671 was isolated from a pine forest soil collected at Hamsterley Forest, County Durham, UK. The almost complete 16S rRNA gene sequence of strain AK 671 was compared with corresponding sequences of representatives of the genus *Streptomyces*. The resulting data indicated that the organism forms a distinct phyletic line in the 16S rRNA *Streptomyces* gene tree and hence probably belongs to a new species.

**Culture conditions:** Fermentations of the strain were performed in a 500-mL fermenter equipped with a magnetic stirrer agitation system (home made) at 800 rpm and an aeration rate of 0.5 vvm at 27 °C in a medium consisting of (per litre tap water) starch soluble 10 g, glucose 10 g, glycerol 10 g, cornsteep powder 2.5 g (Marcor), Bacto peptone 5 g, yeast extract 2 g (Ohly Kat), NaCl 1 g, and CaCO<sub>3</sub> 3 g; pH was adjusted to 7.3 prior to sterilization. The fermenter was inoculated with 5 vol-% of a shake flask culture grown in the same medium for 72 h.

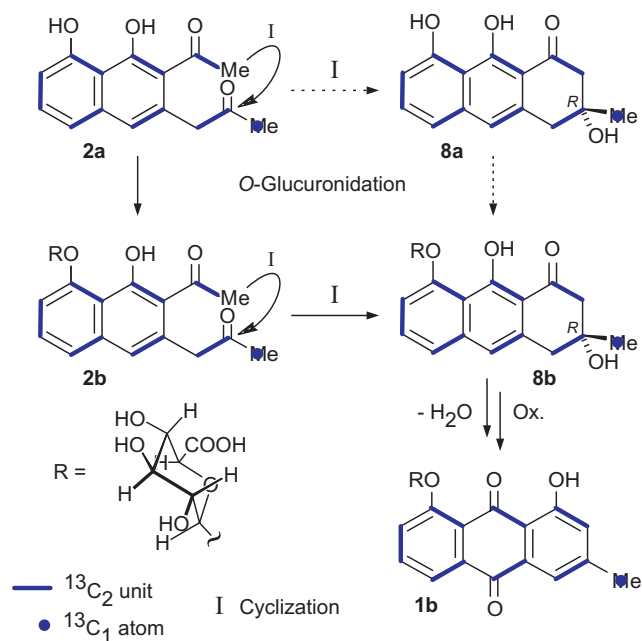
**Feeding experiments and isolation of labeled 1b:** Feeding started after 39 h of incubation. A sterile-filtered solution of 500 mg of <sup>13</sup>C<sub>2</sub>-labeled acetate, dissolved in 20 mL H<sub>2</sub>O, adjusted to pH 7.0, was fed continuously over a period of 10 h. After an incubation period of 160 h in total, the culture broth was centrifuged, the biomass discarded, and the supernatant (350 mL) was applied to an Amberlite XAD-16 column (1.5 × 30 cm). After washing the column with H<sub>2</sub>O, chrysophanol glucuronide (**1b**) was desorbed with H<sub>2</sub>O–MeOH (6:4). After concentration to the aqueous residue *in vacuo* and adjusting to pH 2.0, **1b** was extracted with 1-BuOH. Compound **1b** was obtained in a pure form in a yield of 11 mg by chromatography on Sephadex LH-20 (1.5 × 90 cm) using MeOH as the eluent. The spectroscopic data and chromatographic properties of **1b** were in full accordance with those previously reported.<sup>[14]</sup>

**Feeding experiments and isolation of labeled 2a and 2b:** Feeding started after 24 h of incubation. A sterile-filtered solution of 500 mg of <sup>13</sup>C<sub>2</sub>-labeled acetate, dissolved in 20 mL H<sub>2</sub>O, adjusted to pH 7.0, was fed continuously over a period of 10 h. After an incubation period of 42 h in total, the culture broth was centrifuged, the biomass was discarded, and the supernatant (370

mL) was adjusted to pH 2.0 and extracted three times with EtOAc. Compounds **2a** and **2b** were obtained in a pure form in yields of 16 mg and 10 mg, respectively, by chromatography on Sephadex LH-20 (1.5 × 90 cm) using MeOH as the eluent. The spectroscopic data and chromatographic properties of **2a** and **2b** were in full accordance with those previously reported.<sup>[14]</sup>

**NMR spectroscopy:** NMR spectra were recorded on a Bruker DMX 600 spectrometer. For calibration of <sup>13</sup>C and <sup>1</sup>H chemical shifts, the methyl-carbon signal and the residual proton signal of the solvent were used, respectively (methanol-*d*<sub>4</sub>: δ<sub>H</sub> = 3.31 ppm and δ<sub>C</sub> = 49.1 ppm). To establish <sup>13</sup>C-<sup>13</sup>C connectivities, 2D INADEQUATE experiments were performed at 150.9 MHz using a carbon-sensitive cryoprobe.

**Scheme S-1** Biosynthetic relationship of **2a**, its glucuronide **2b**, prechrysoanol **8a** and its glucuronide **8b**, and the final product, chrysoanol glucuronide (**1b**).



**Table S-1.**  $^{13}\text{C}$ -NMR data of chrysophanol glucuronide (**1b**).  $^{13}\text{C}$  NMR chemical shifts and enrichment ratios of labeled positions of **1b** isolated after feeding of  $^{13}\text{C}_2$ -acetate. The enrichment is given as atom%  $^{13}\text{C}$  exceeding the natural abundance.

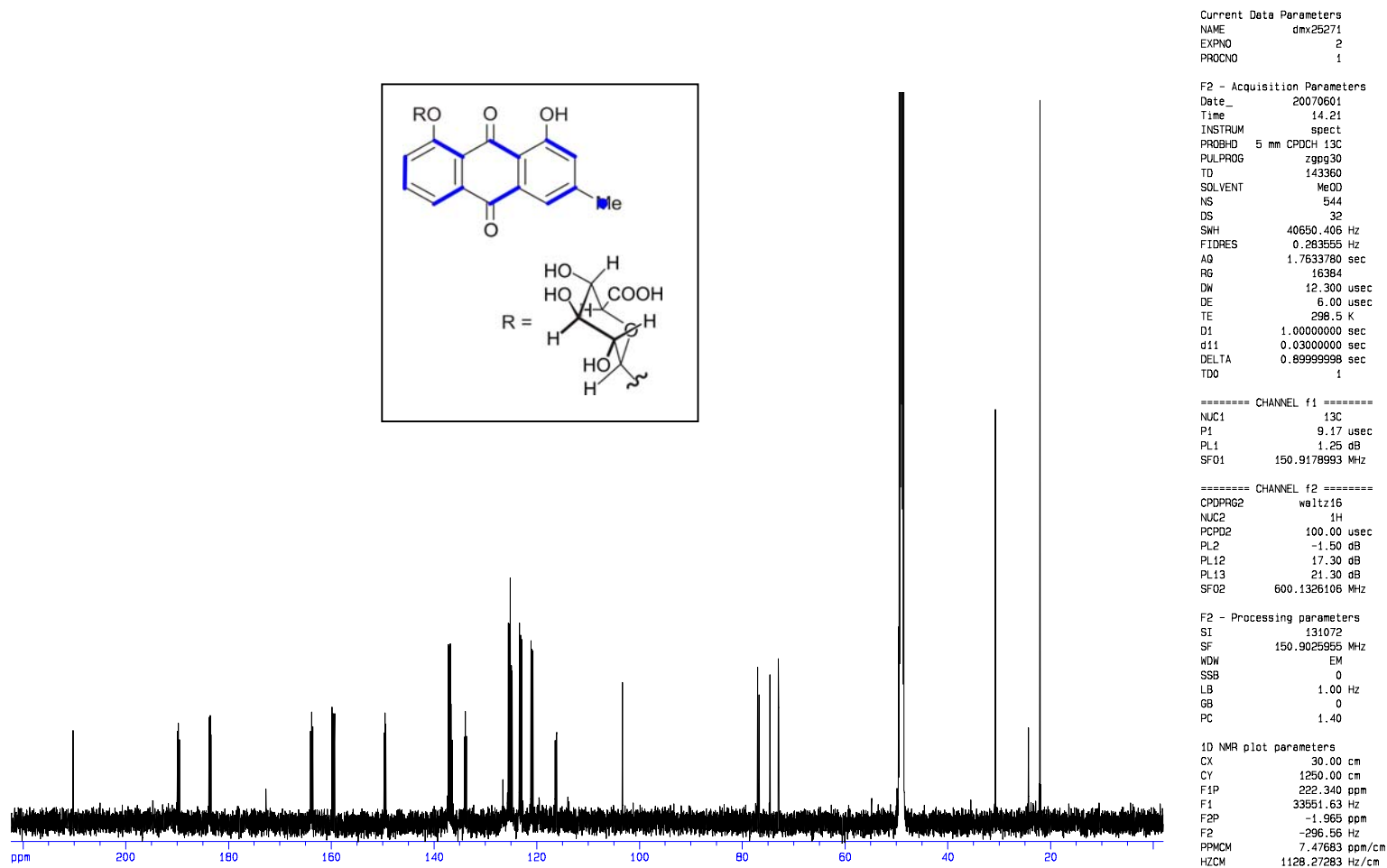
Carbon	$\delta$ [ppm]	Enrichment [%]	$^2J$ (to C)
1	163.9	1.1	67.1 (2)
2	125.4	1.1	66.9 (1)
3	149.7	1.3	55.6 (4)
4	121.1	1.3	55.7 (3)
4a	134.0	1.1	54.3 (10)
10	183.7	1.1	54.2 (4a)
10a	136.8	1.1	60.3 (5)
5	123.3	1.1	60.5 (10a)
6	137.1	1.2	55.9 (7)
7	125.0	1.2	55.8 (6)
8	159.7	1.4	70.5 (8a)
8a	123.3	1.4	70.5 (8)
9	189.9	1.2	56.0 (9a)
9a	115.5	1.2	56.0 (9)
3-Me	22.2	n.d.	s

**Table S-2.**  $^{13}\text{C}$ -NMR data of genoketide A2 (**2b**).  $^{13}\text{C}$  NMR chemical shifts and enrichment ratios of labeled positions of **2b** isolated after feeding of  $^{13}\text{C}_2$ -acetate. The enrichment is given as atom%  $^{13}\text{C}$  exceeding the natural abundance.

Carbon	$\delta$ [ppm]	Enrichment [%]	$^2J$ (to C)
1	153.5	2.6	74.9 (2)
2	125.5	2.6	77.2 (1)
3	133.0	3.6	63.1 (4)
4	122.1	3.6	62.8 (3)
4a	137.4	2.7	55.6 (5)
5	124.0	2.7	55.6 (10)
6	128.5	3.8	54.5 (7)
7	112.2	3.8	55.1 (6)
8	155.6	3.7	55.9 (8a)
8a	115.4	3.7	55.8 (8)
9	204.7	3.6	42.7 (10)
10	32.0	3.6	42.5 (9)
11	48.4	2.9	37.9 (12)
12	205.4	2.9	37.9 (11)
13	29.9	n.d.	s

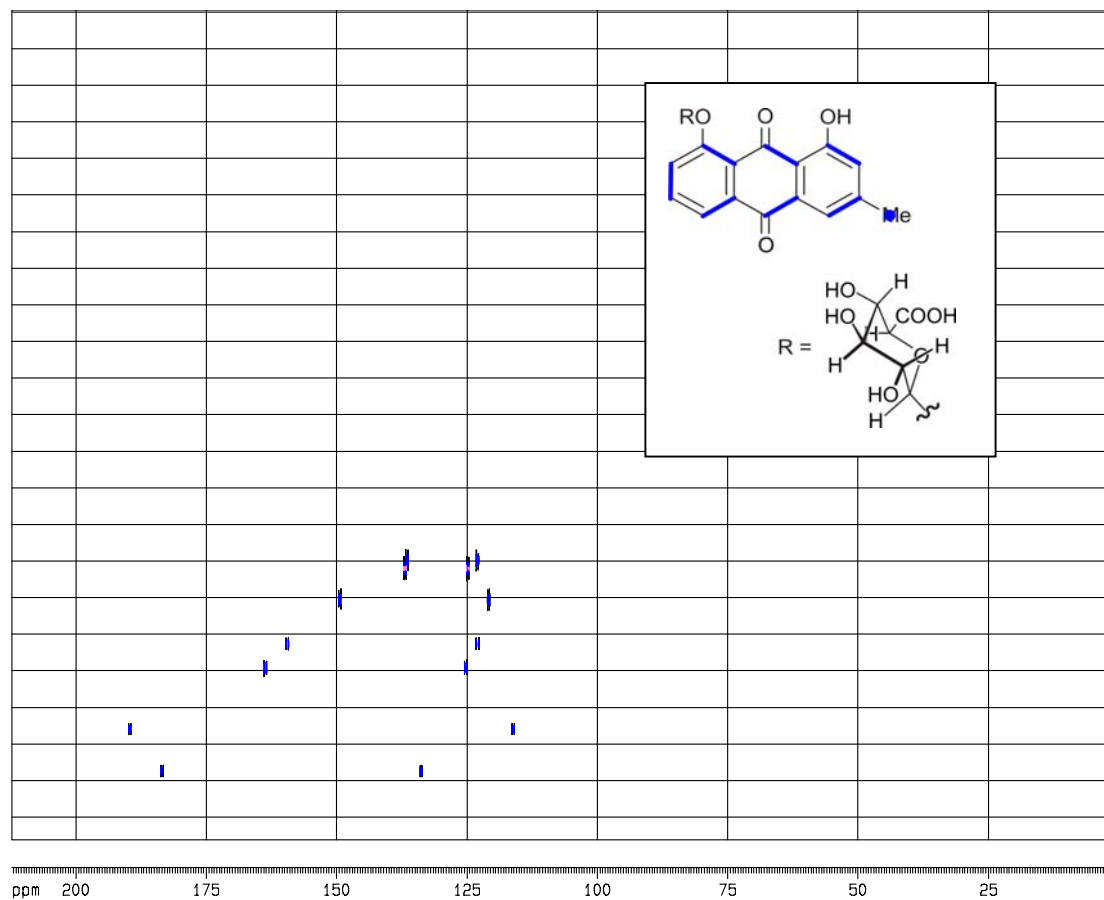
## Figure S-2. $^{13}\text{C}$ NMR spectrum of chrysophanol glucuronide (**1b**)

Tobias Gulder, AK-671 T - %C13CPD\_de, 13C, 150.9 MHz



**Figure S-3.** 2D INADEQUATE spectrum of chrysophanol glucuronide (**1b**)

Tobias Guider, AK-671 T - %INAD, 2D-INADEQUATE, 150.9 MHz



```
Current Date Parameters
NAME          dmx25271
EXPNO         3
PROCNO        1

F2 - Acquisition Parameters
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PULPROG       inadqf.2
TD            32768
SOLVENT       MeOD
NS            192
DS            64
SWH           34482.758 Hz
FIDRES        1.052330 Hz
AQ            0.4751850 sec
RG            32768
DW            14.500 usec
DE            6.00 usec
TE            298.5 K
CNST3         50.000000
d0            0.0000000 sec
d1            6.0000000 sec
d4            0.0000000 sec
d11           0.0300000 sec
IN0           0.0001450 sec

***** CHANNEL f1 *****
NUC1          13C
P1            9.17 usec
p2            18.34 usec
PL1           1.25 dB
SF01          150.9190190 MHz

***** CHANNEL f2 *****
CPDPRG2       waltz16
NUC2          13H
PCPD2         100.00 usec
PL2           -1.50 dB
PL12          17.30 dB
SF02          500.1326105 MHz

F1 - Acquisition parameters
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SF01          150.919 MHz
FIDRES        459.770111 Hz
SW            456.970 ppm
FMODE         GF

F2 - Processing parameters
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SF            150.9025965 MHz
WDW           GSIINE
SSB           2
LB            0.00 Hz
GB            0
PC            1.00

F1 - Processing parameters
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MC2           GF
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WDW           SINE
SSB           2
LB            0.00 Hz
GB            0

2D NMR plot parameters
CX2           20.00 cm
CX1           15.00 cm
F2PLO         212.337 ppm
F2FLO         320.215 Hz
F2PHI         -0.150 ppm
F2HI          -28.66 Hz
F1PLO         336.142 ppm
F1FLO         50719.24 Hz
F1PHI         109.393 ppm
F1HI          16505.88 Hz
F2PPMCM       10.52633 ppm/cm
F1PPMCM       100.00000 ppm/cm
```