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Supporting Information

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for

Regio- and Stereoselective Intermolecular Oxidative Phenol Coupling in Kotanin Biosynthesis by *Aspergillus niger*

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1. ^{13}C and relevant ^{1}H NMR-spectra of ^{13}C-enriched bicoumarins

1.1 First feeding experiment: [2-^{13}C]-dihydroxycoumarin ^{13}C-6

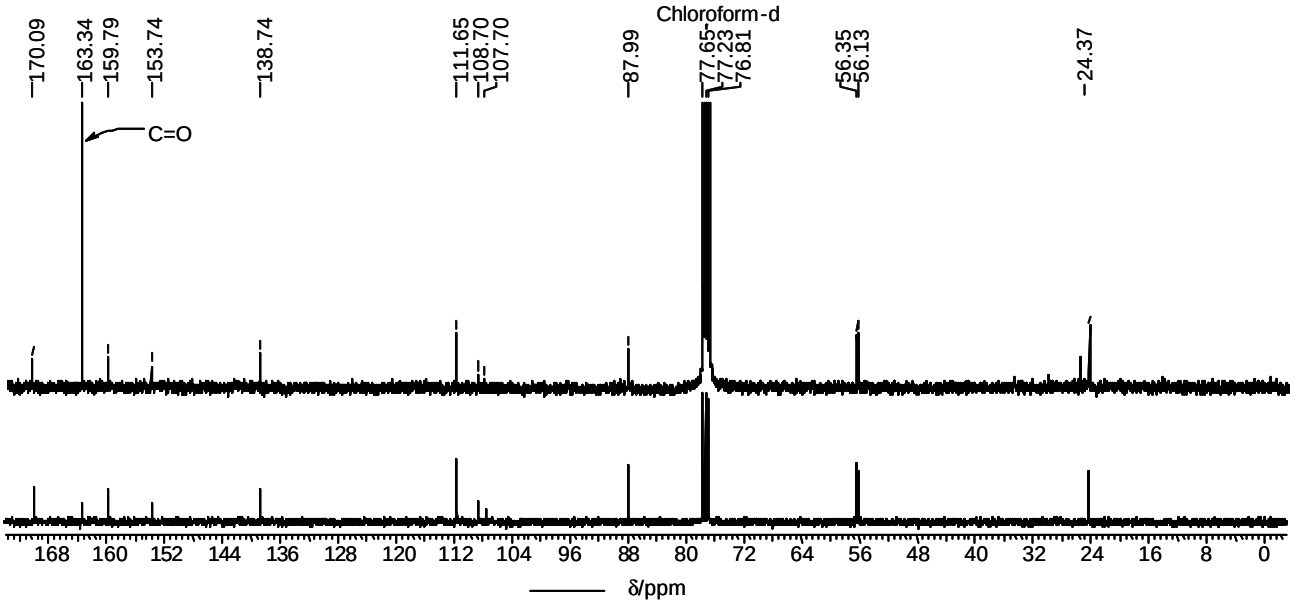


Figure S1. ^{13}C **NMR**-spectra of kotanin (1) isolated from *A. niger* after feeding of [2-^{13}C]-dihydroxy-cumarin ^{13}C-6 (top) and without feeding (below); $CDCl_3$, 75 MHz.

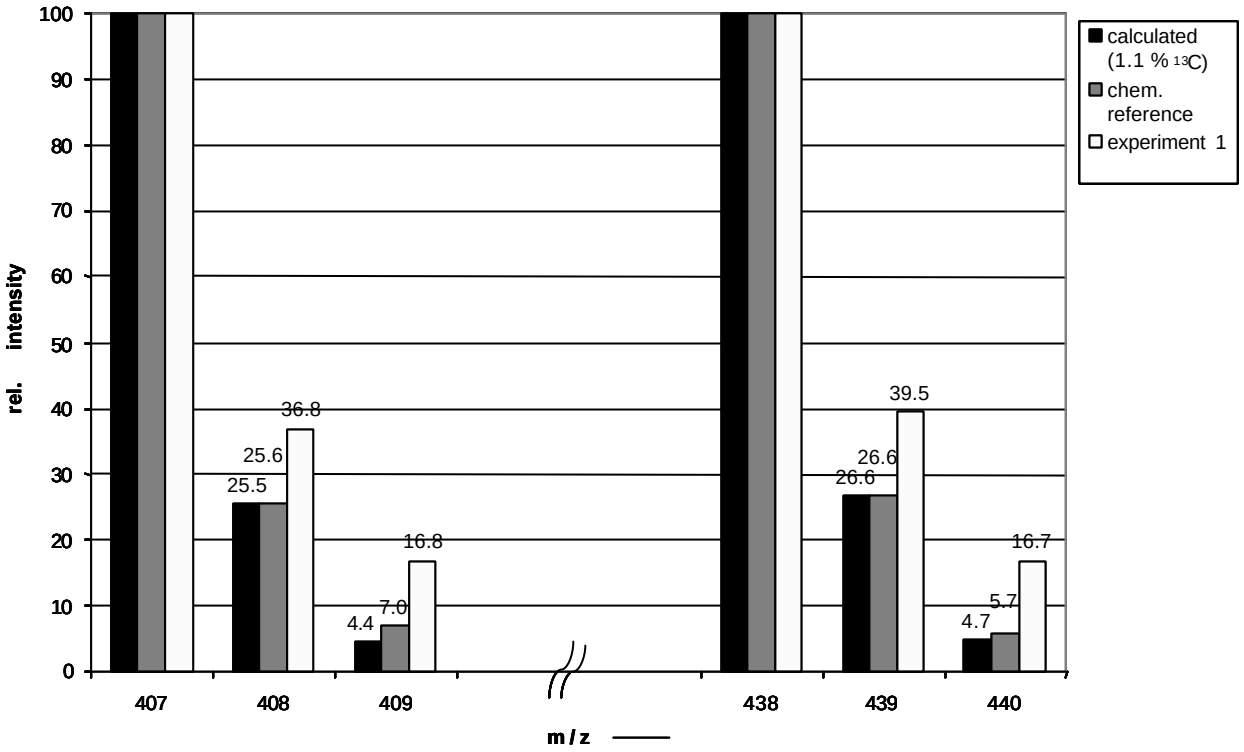


Figure S2. Intensities of the peaks M^+ (438) and M^+ -OCH_3 (407) as well as the corresponding isotope peaks M+1 and M+2 in mass spectra of kotanin (1). To the main peaks (438 und 407) an intensity $I \equiv$ 100 was assigned.

1.2 Second feeding experiment: [2-^{13}C]-demethylsiderin [^{13}C-5]

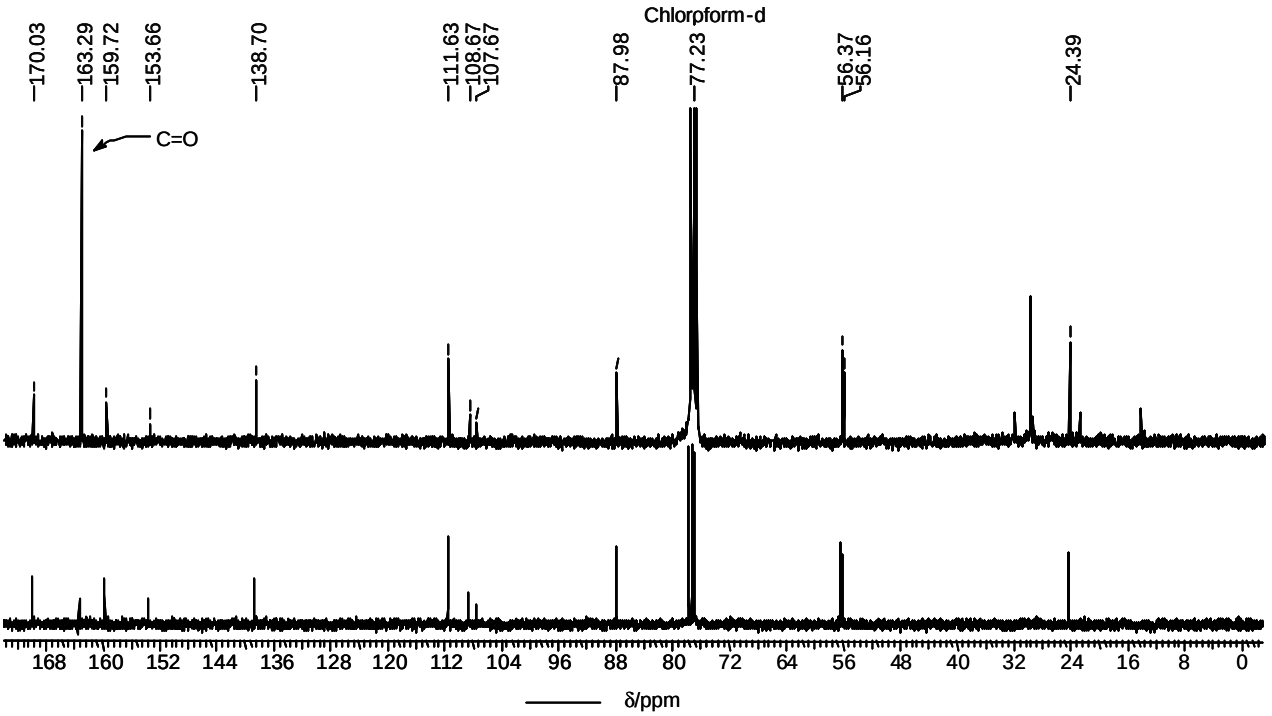


Figure S3. ^{13}C **NMR**-spectra of kotanin (1) isolated from *A. niger* after feeding of [2-^{13}C]-demethylsiderin (^{13}C-5) (top) and without feeding (below); $CDCl_3$, 75 MHz.

1.3 Third feeding experiment: [2-^{13}C,7-O^{13}CH$_3$]-siderin [2×^{13}C-4]

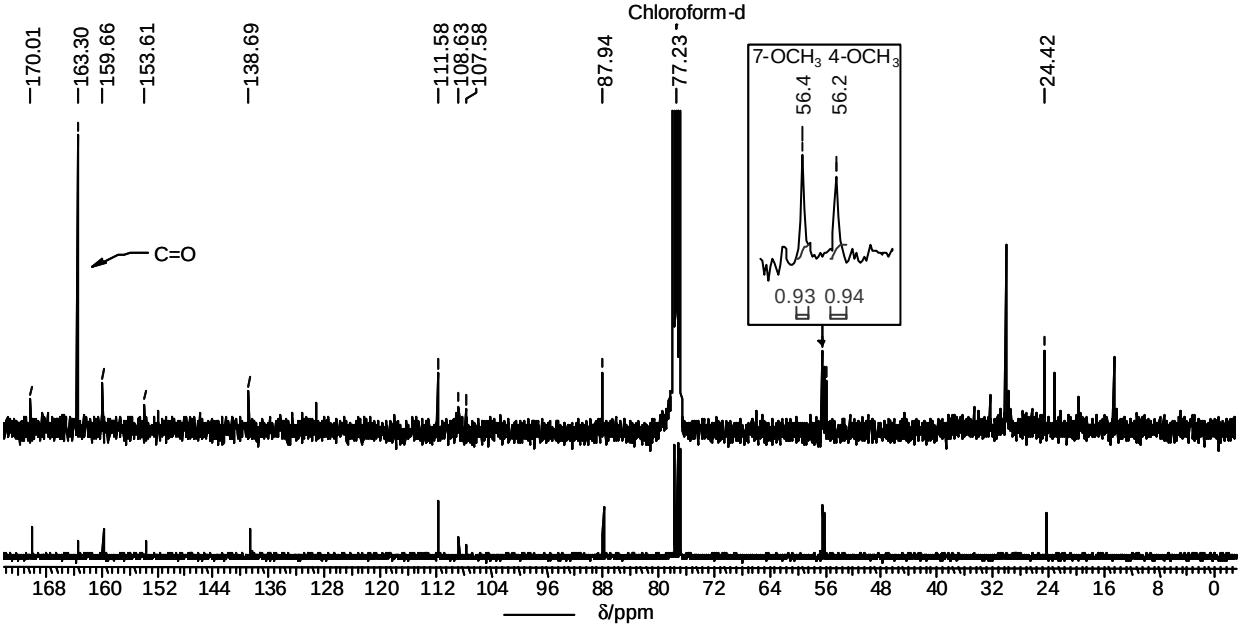


Figure S4. ^{13}C **NMR**-spectra of kotanin (1) isolated from *A. niger* after feeding of [2-^{13}C,7-O^{13}CH$_3$]-siderin (2×^{13}C-4) (top) and without feeding (below); $CDCl_3$, 75 MHz.

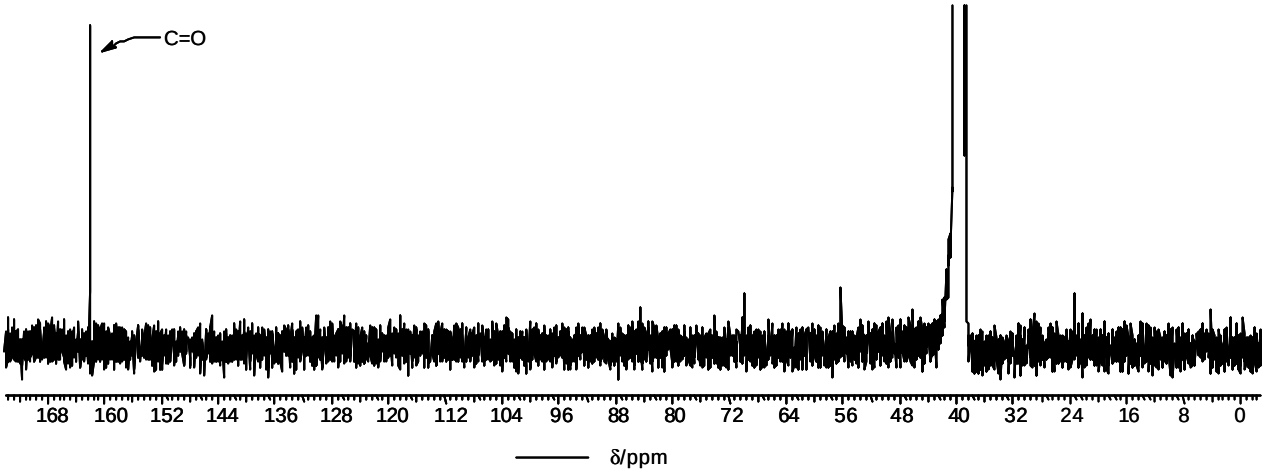


Figure S5. ^{13}C **NMR**-spectrum of orlandin (10) isolated from *A. niger* after feeding of [2-^{13}C,7-O$^{13}CH_3$]-siderin (2×^{13}C-4), $CDCl_3$, 75 MHz.

1.3 Fourth feeding experiment: [2-^{13}C,4-O$^{13}CH_3$]-demethylsiderin [2×^{13}C-5]

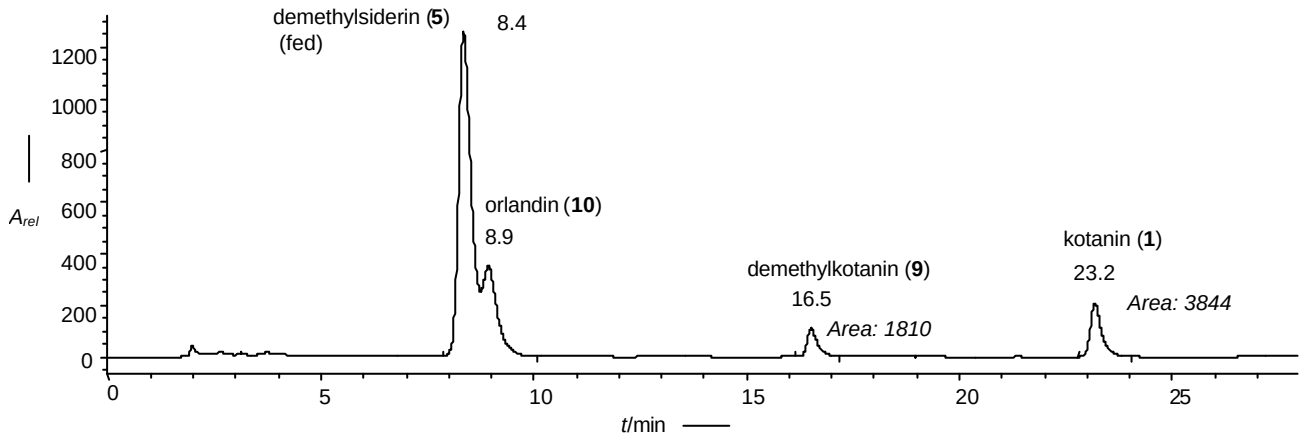


Figure S6. HPLC-chromatogramm (RP-18) of the crude extract of feeding experiment 4 (20 m**L** chloroform extract were prepared from 400 m**L** culture medium).

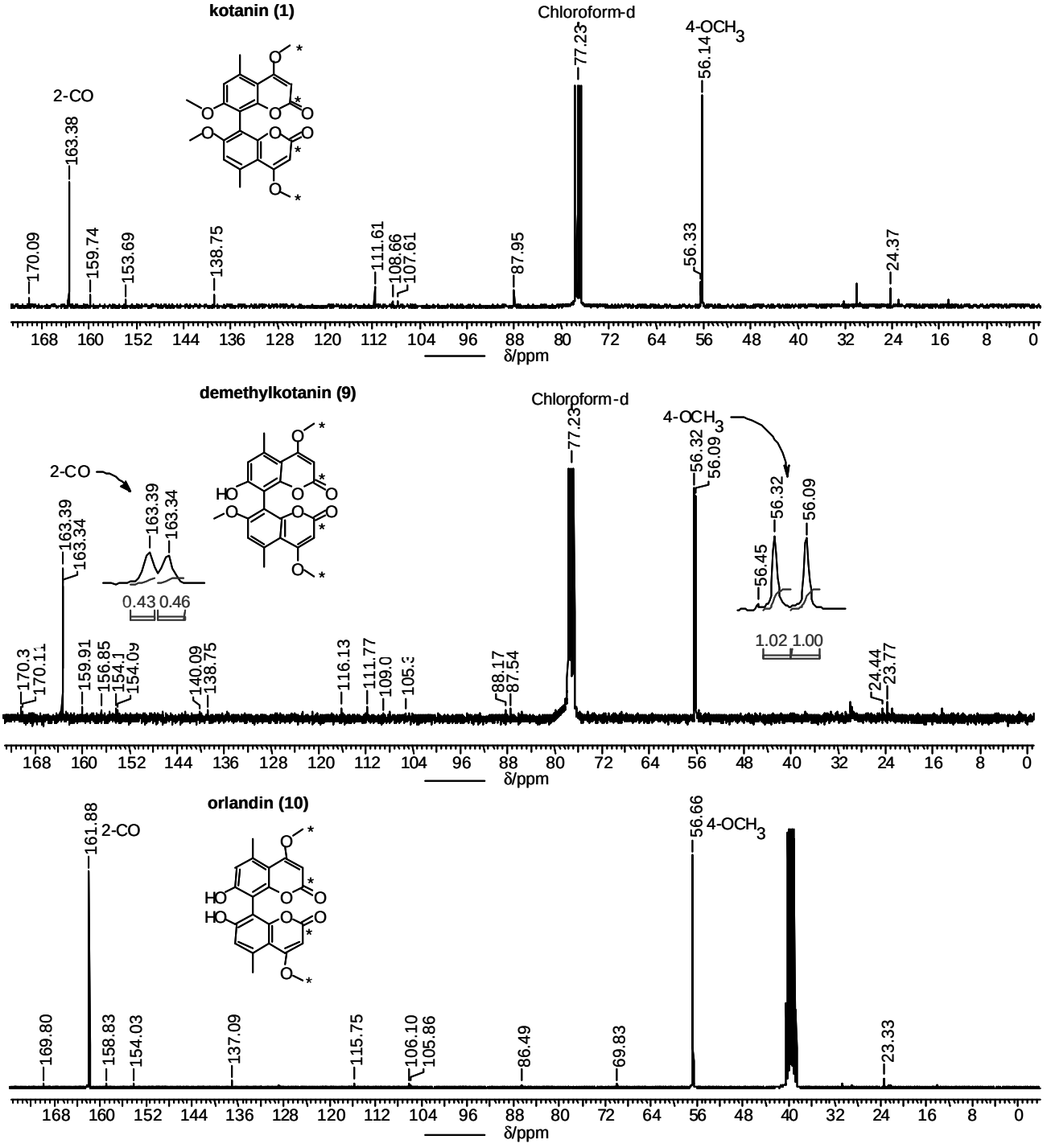


Figure S7. ^{13}C **NMR**-spectra of bicoumarins isolated from *A. niger* after feeding of [2-^{13}C,4-O$^{13}CH_3$]-demethylsiderin (2×^{13}C-4); $CDCl_3$ (kotanin (1) and demethylkotanin (9)) and [D_6]DMSO (orlandin (10)); 75 MHz.

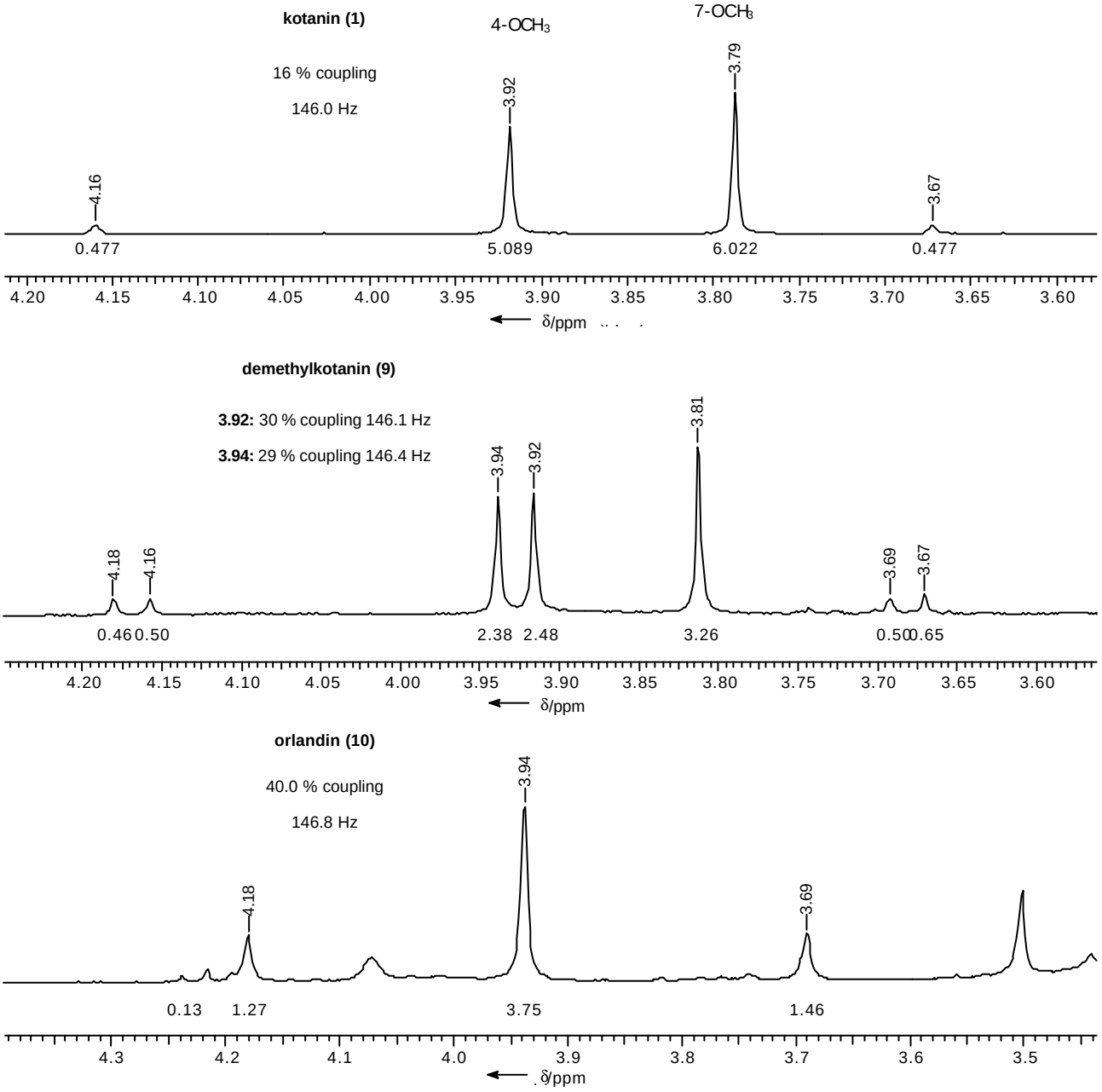
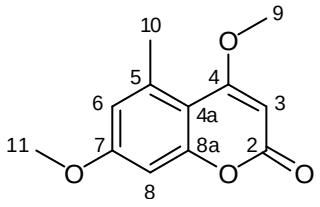


Figure S8. OCH_3-signals in ^1H NMR-spectra of bicoumarins isolated from *A. niger* after feeding of [2-^{13}C,4-O$^{13}CH_3$]-demethylsiderin (2×^{13}C-4); $CDCl_3$ (kotanin (1) and demethylkotanin (9)) and **[D$_6$]**DMSO (orlandin (10)); 300 MHz.

2. Relative ^{13}C NMR-signal intensities of monomeric coumarins.

Table S1. Relative intensities of ^{13}C NMR signals. Relative intensity = integral of signal / integral of signal C-10;



	Position											
compound	**10**	**11**	**9**	**3**	**8**	**4a**	**6**	**5**	**8a**	**7**	**2**	**4**
siderin (4)[a, c] reference	1.00	0.90	0.95	0.96	1.01	0.19	1.02	0.33	0.25	0.36	0.32	0.28
2×^{13}C-siderin (2×^{13}C-4)[a, c]	1.00	97.98	2.21[e]	0.88	0.98	0.21	1.02	0.42	0.29	0.41	29.99	0.34
2×^{13}C-siderin (2×^{13}C-4)[b, c]	1.00	98.52	2.15[e]	0.87	1.02	0.17	0.96	0.37	0.28	0.39	27.53	0.30
demethylsiderin (5)[a, d], reference	1.00	-	1.02	0.76	0.91	0.30	0.85	0.45	0.34	0.58	0.58	0.44
^{13}C-demethyl-siderin (^{13}C-5)[a, d]	1.00	-	0.88	0.86	1.01	0.32	0.93	0.54	0.52	0.65	44.62	0.40
^{13}C-demethyl-siderin (^{13}C-5) exp. 3[b, d]	1.00	-	1.71[e]	069	0.83	0.36	0.85	0.62	0.52	0.65	44.45	0.53
2×^{13}C-demethyl-siderin (2×^{13}C-5)[a, d]	1.00	-	109.78	0.87	1.08	0.43	1.07	0.57	0.56	0.65	50.09	0.53

[a] from chemical synthesis, [b] isolated from *A. niger*, [c] $CDCl_3$, [d] [D_6]DMSO, [e] minor ^{13}C enrichment after acid catalyzed methyl group exchange during synthesis.

3. Alternative HPLC-method for crude extract analysis (normal phase)

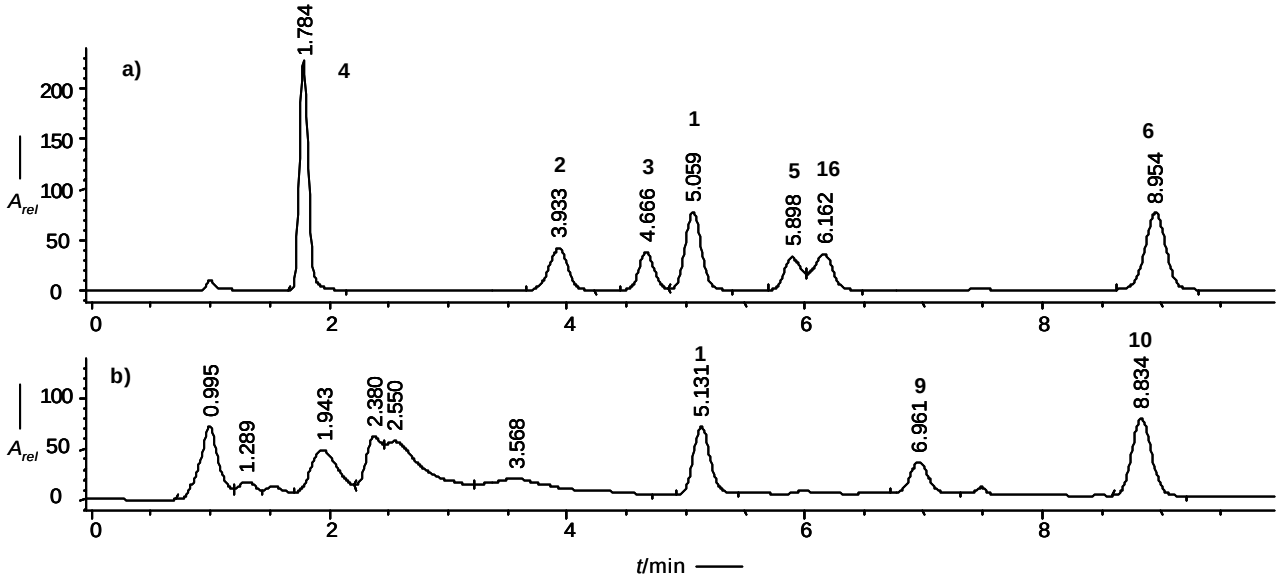


Figure S9. Alternative HPLC-analysis of *A. niger* crude extract. a) chemically synthesized reference compounds; b) crude extract; compounds: kotanin (**1**), isokotanin A (**2**), desertorin C (**3**), siderin (**4**), demethylsiderin (**5**), 3,7-dihydroxy-4-methylcoumarin (**6**), demethylkotanin (**9**), orlandin (**10**) and 4-hydroxy-7-methoxy-5-methylcoumarin (**16**); HP 1100 HPLC System (Agilent), Lichrospher® Si60 column (250 × 3 mm, 5 µm particle size; CS Chromatographie Service GmbH, Langerwehe, Germany); precolumn (20 × 3 mm) of the same stationary phase, 25 °C, elution gradient: MeOH/$CHCl_3$ (1 % formic acid), 0:100 ? 5:95 in 10 min, flow rate: 1.5 mL min^{-1}), detection wavelength: 308 nm.

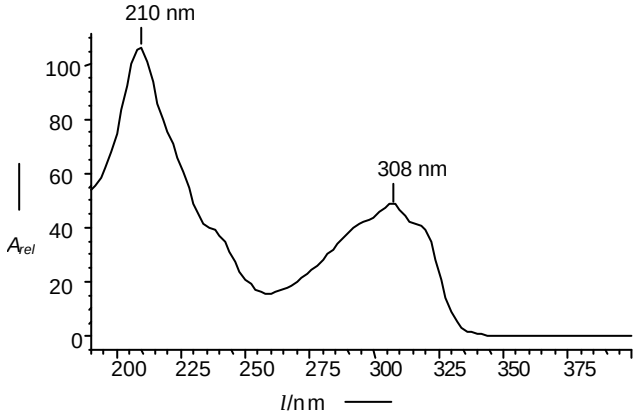


Figure S10. UV-spectrum of kotanin (**1**) measured with the HPLC-DAD-detector. The absorption pattern is characteristic for all siderin-type coumarins.

4. CD-spectra of bicoumarins isolated from *A. niger*

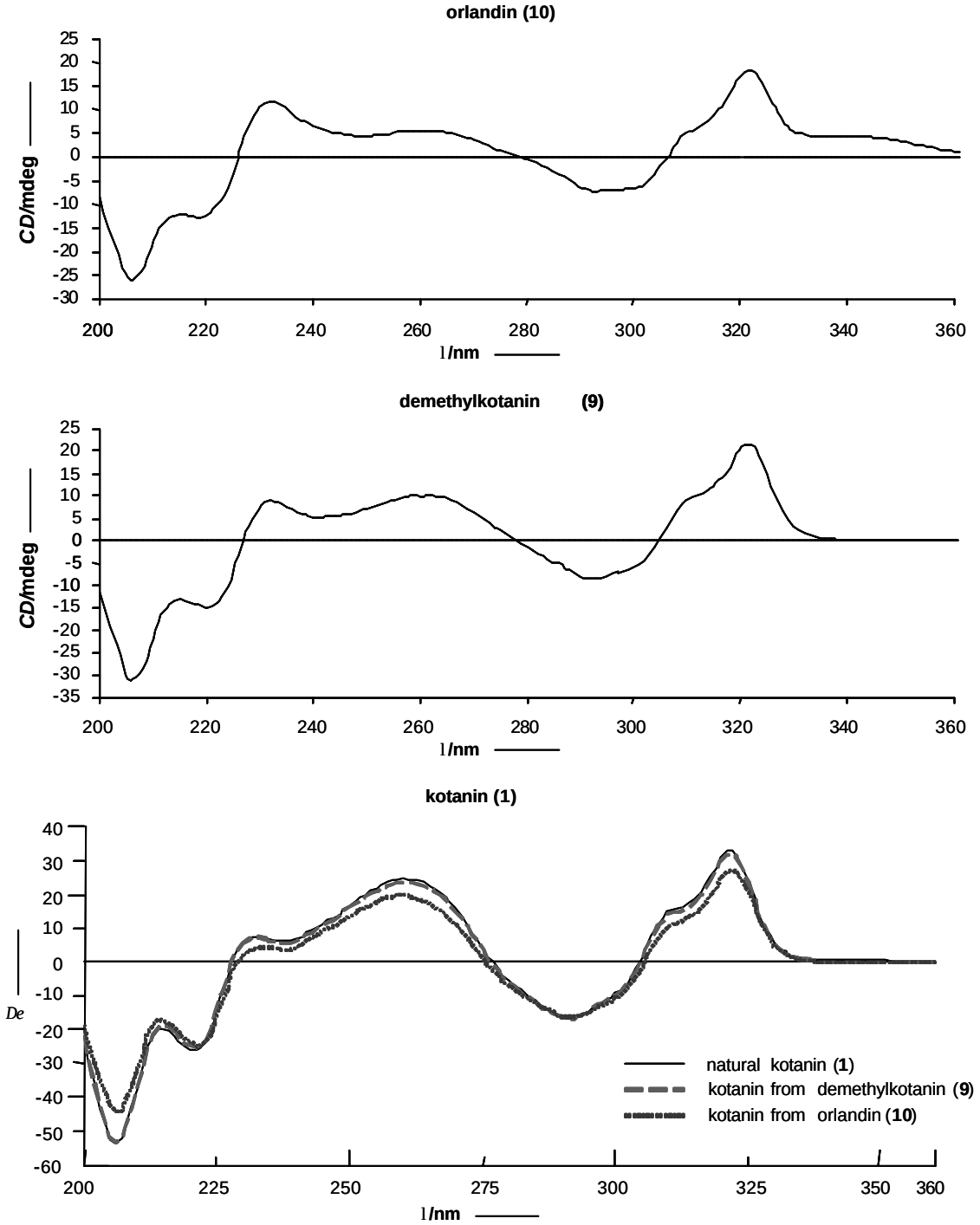


Figure S11. CD-spectra of orlandin (**10**), demethylkotanin (**9**) and kotanin (**1**) isolated from *A. niger* as well as spectra of kotanin (**1**) obtained by *O*-methylation of demethylkotanin (**9**) and orlandin (**10**). (acetonitrile, 1 % MeOH in case of orlandin (**10**)). The circular-dichroic absorptions $\Delta\varepsilon$ are reported in ° cm^2 $dmol^{-1}$. Since the concentrations of 10 and 9 cannot be determined precisely, a device-specific unit is shown in the intensity axis.