# Anti-inflammatory dihydroxanthones from a *Diaporthe* species

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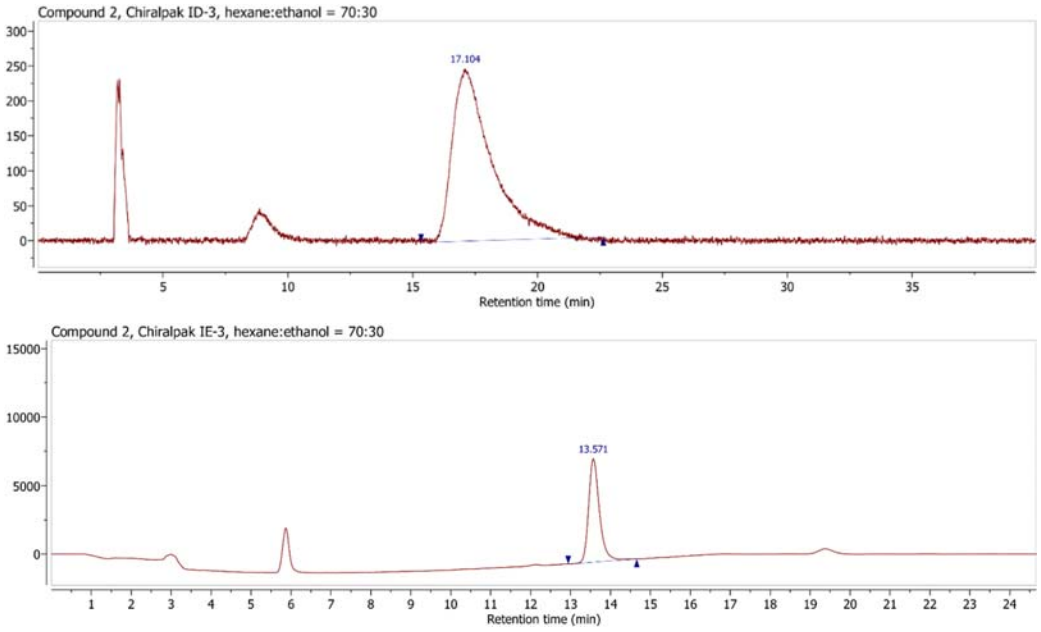
## Supplementary material

### Chiral HPLC Analysis

A chiral HPLC analysis (normal phase, isocratic mode with ethanol:*n*-hexane = 70:30) was performed using Chiralpak ID-3, IE-3, IF-3 columns (Daicel Corporation) as well as an Infinity Lab Poroshell 120 Chiral-V column (Agilent). Due to the limited stability of compound **2** under these conditions, dehydration at C-1/C-2 proceeded (see total chromatograms below). The degradation product (the less polar peak) was identified via LC-MS analysis (Figure S1).



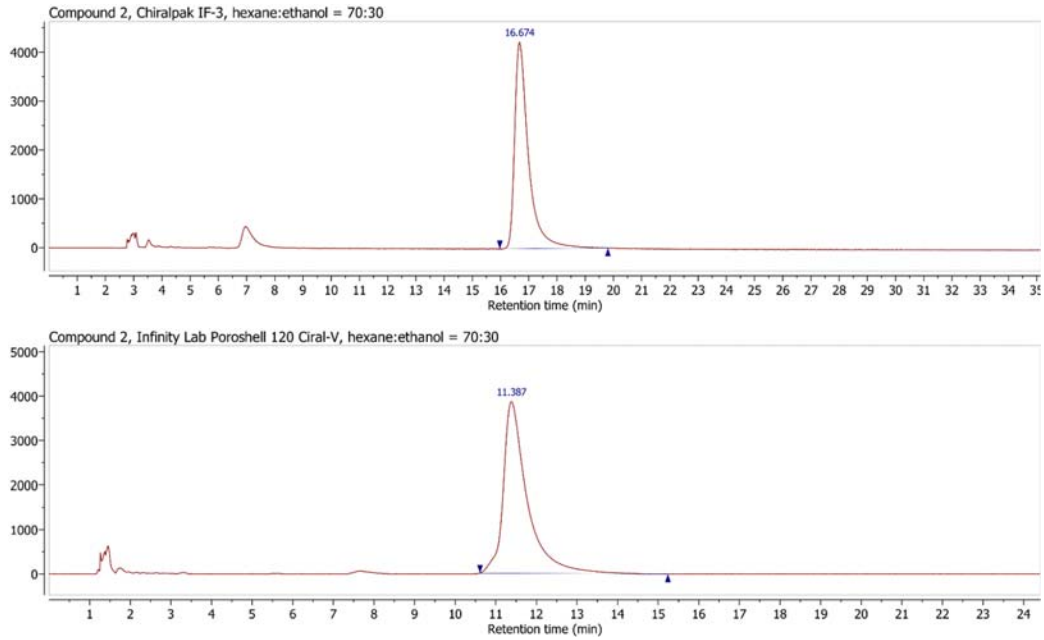



*Figure S1: Total Chromatograms of compound **2** using a Chiralpak ID-3, IE-3, IF-3 column as well as an Infinity Lab Poroshell 120 Chiral-V column in isocratic mode with ethanol:n-hexane = 70:30.*

When a Chiralpak IA-3 column (mobile phase: *n*-hexane:EtOH = 85:15, 1 mL $min^{-1}$, λ =190-400 nm, 40 °C) was used, chromatograms of compound **2** revealed an additional peak in the trailing flank of the main signal (Figure S2). The signals at 37 min and 40 min retention time showed no difference in their UV spectra and therefore the second signal may corresponded to the enantiomer of compound **2** although this remains uncertain. The depicted chromatograms display the best separation that could be obtained. The tangent skim integration method was used for the determination of the hypothetical *enantiomeric excess* = 76% (*enantiomeric ratio* = 7.3:1). The area percentages are given in Table S1.

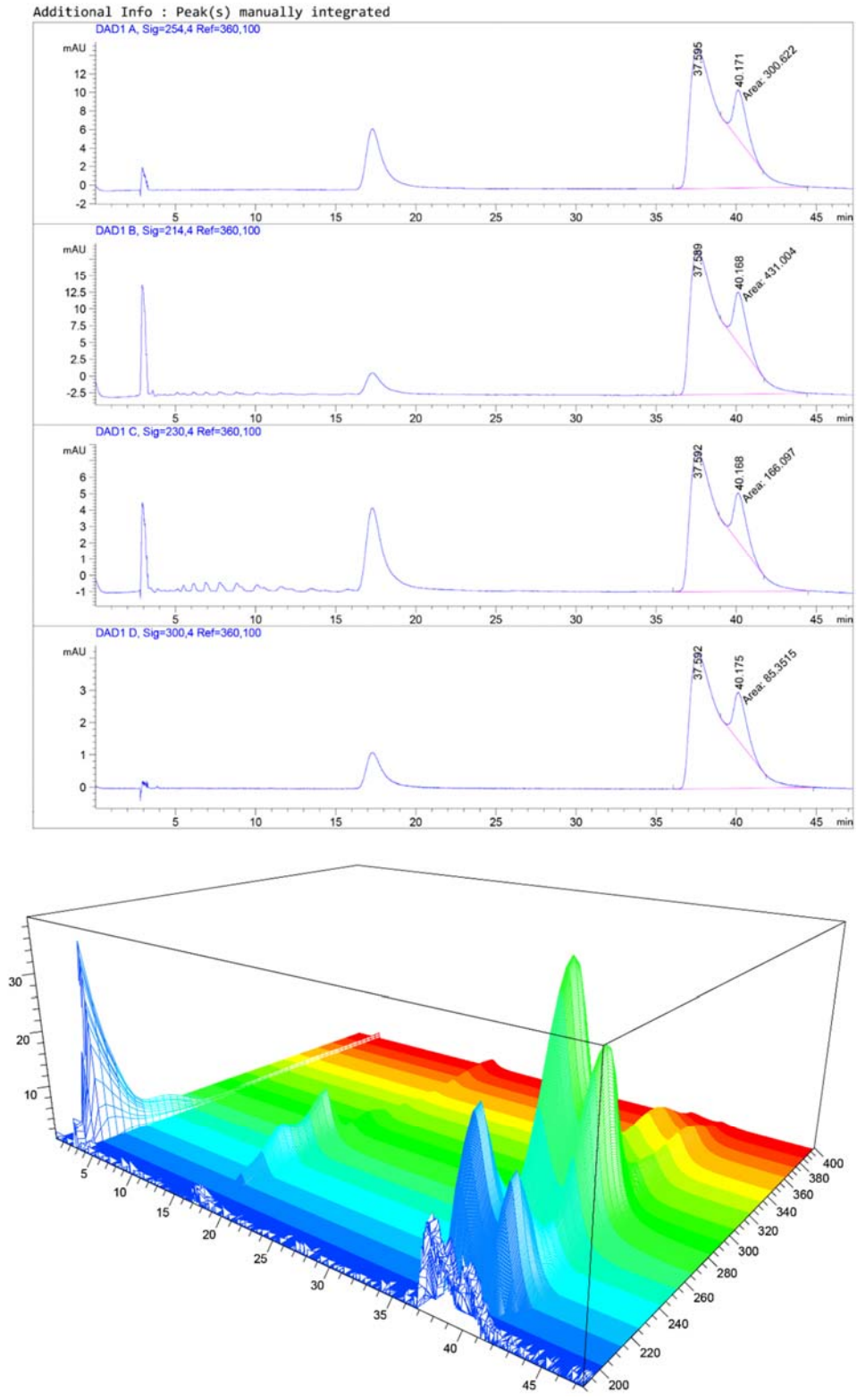



*Figure S2: DAD traces of compound **2** using a Chiralpak IA-3 column in isocratic mode with ethanol:n-hexane = 85:15.*

*Table S1: The area percent report of the respective DAD signals of compound **2**.*

| | Peak # | Retention Time [min] | Width [min] | Area [mAU*s] | Height [mAU] | Area % |
|---|---|---|---|---|---|---|
| Signal: 254 nm | 1 | 37.595 | 2.2924 | 2333.21509 | 15.02205 | 88.5862 |
| | 2 | 40.171 | 1.0020 | 300.62195 | 5.33489 | 11.4138 |
| Signal: 214 nm | 1 | 37.589 | 2.5778 | 3336.38647 | 21.57160 | 88.5596 |
| | 2 | 40.168 | 1.0016 | 431.00433 | 7.68469 | 11.4404 |
| Signal: 230 nm | 1 | 37.592 | 2.5989 | 1334.82800 | 8.56030 | 88.9337 |
| | 2 | 40.168 | 0.9246 | 166.09726 | 2.99395 | 11.0663 |
| Signal: 300 nm | 1 | 37.592 | 2.5828 | 653.94946 | 4.21996 | 88.4551 |
| | 2 | 40.175 | 0.9428 | 85.35149 | 1.50882 | 11.5449 |

If compound **2** was indeed a scalemic mixture, the determination of the absolute configuration of the major component would still be valid: Division of the experimental $[\alpha]_D$ of compound **2** ($[\alpha]_D^{29}$ –72.6 (c = 0.2, MeOH)) by the putative enantiomeric excess, the hypothetical optical rotation of the pure enantiomer (–)-**2** can be estimated ($[\alpha_{est.}]_D^{29}$ –95.5 (c = 0.2, MeOH)). The difference of this value and the calculated value of the *RR* enantiomer lies within Stephens' error margin of 57.8 (–46.3 – –95.5 = 49.2), whereas the difference with its antipode is > 57.8 (+46.3 – –95.5 = 141.8). Therefore, the AC of the (–)-enantiomer of compound **2** can be assigned with >95% confidence.

## Computational details and input lines for the calculation of chemical shifts and the optical rotation of compound 2.

Conformational analysis was performed using Spartan '10[1] (MMFF level of theory[2]):

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All DFT calculations were performed using Gaussian 16, Rev. A.03.[3] Geometry optimizations with the B3LYP functional[4-7], the Pople basis set 6-31G(d)[8, 9] and the IEFPCM solvation model[10] for methanol:

#p opt=tight freq=noraman b3lyp 6-31g(d) scrf=(iefpcm,solvent=methanol)

NMR calculations for the DP4+-method using the mpw1pw91 functional[11] with the 6-311+G(d,p) basis set[12-14]:

#p nmr= giao mpw1pw91 6-311+g(d,p) scrf= (iefpcm,solvent=methanol)

NMR calculations for the *J*-DP4-method:

#p nmr=(giao,spinspin) b3lyp/6-31g(d,p)

Calculation of the optical rotation using the optrot method[15, 16]and the Dunning basis set aug-cc-pVTZ[17, 18]:

#p temperature=302.15 polar=optrot cphf=rdfreq b3lyp/aug-cc-pVTZ scrf=(iefpcm,solvent=methanol)

Table S2: DP4+-probabilities for the *RR* and the *RS* configuration of compound **2**.

| | | *RR* | *RS* |
|---|---|---|---|
| *s*DP4+ | (H data) | 0.96% | 99.04% |
| *s*DP4+ | (C data) | 99.86% | 0.14% |
| *s*DP4+ | (all data) | 87.14% | 12.86% |
| *u*DP4+ | (H data) | 60.20% | 39.80% |
| *u*DP4+ | (C data) | 99.93% | 0.07% |
| *u*DP4+ | (all data) | 99.95% | 0.05% |
| DP4+ | (H data) | 1.45% | 98.55% |
| DP4+ | (C data) | 100.00% | 0.00% |
| **DP4+** | **(all data)** | **99.99%** | **0.01%** |

Table S3: *J*-DP4-probabilities for the *RR* and the *RS* configuration of compound **2**.

| | *RR* | *RS* |
|---|---|---|
| H | 0.24% | 99.76% |
| C | 5.45% | 94.55% |
| H+C | 0.01% | 99.99% |
| *J* | 62.20% | 37.80% |
| **all data** | **0.02%** | **99.98%** |

## References


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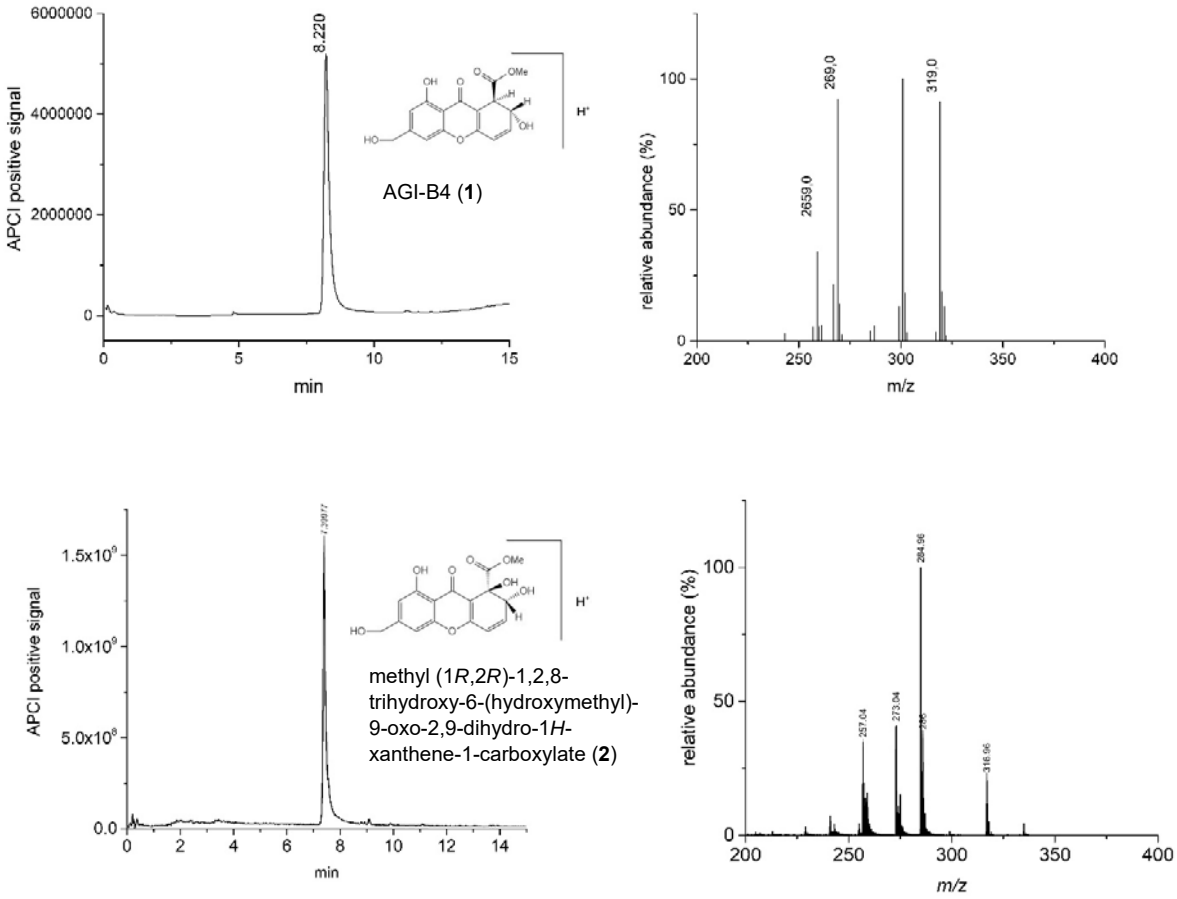
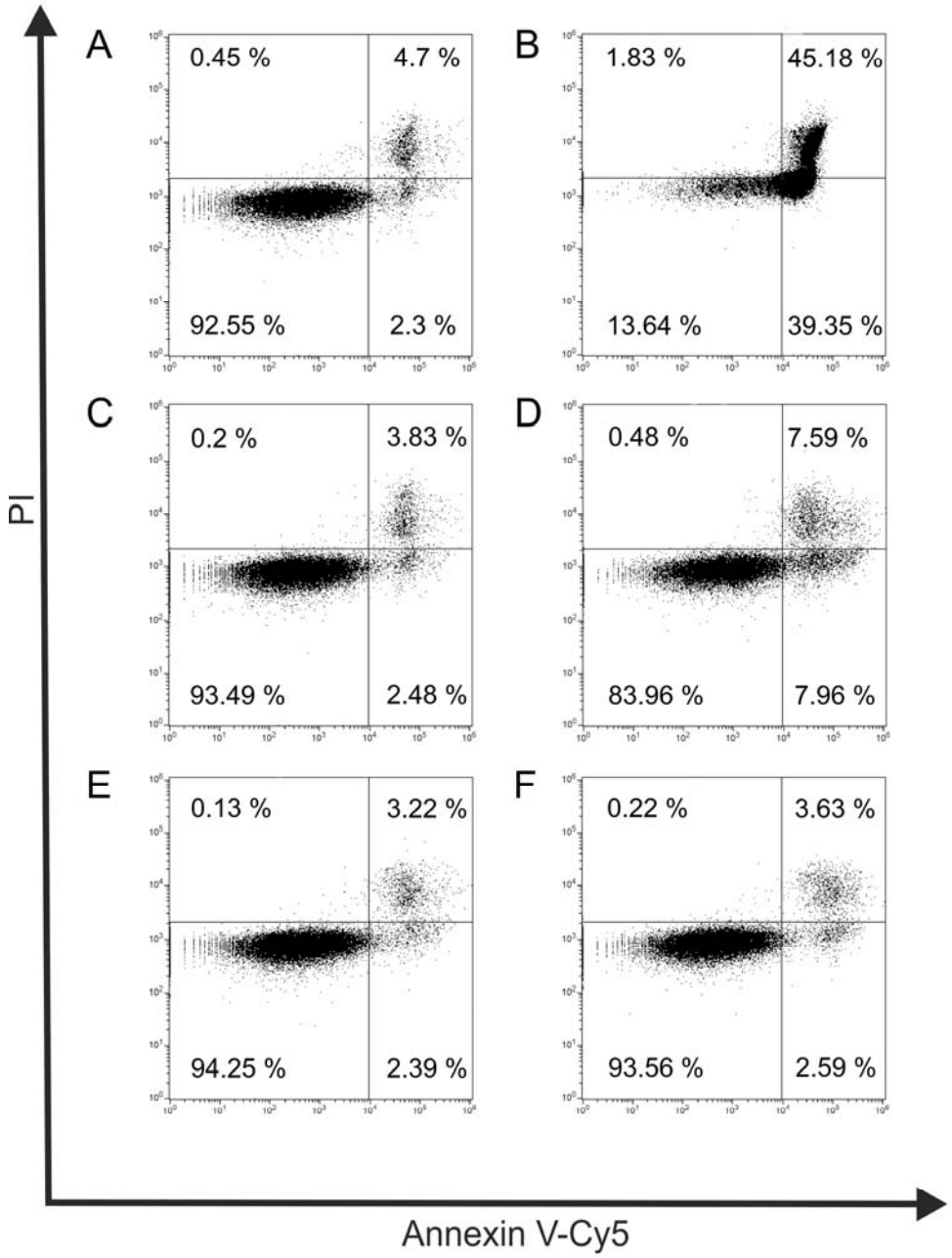


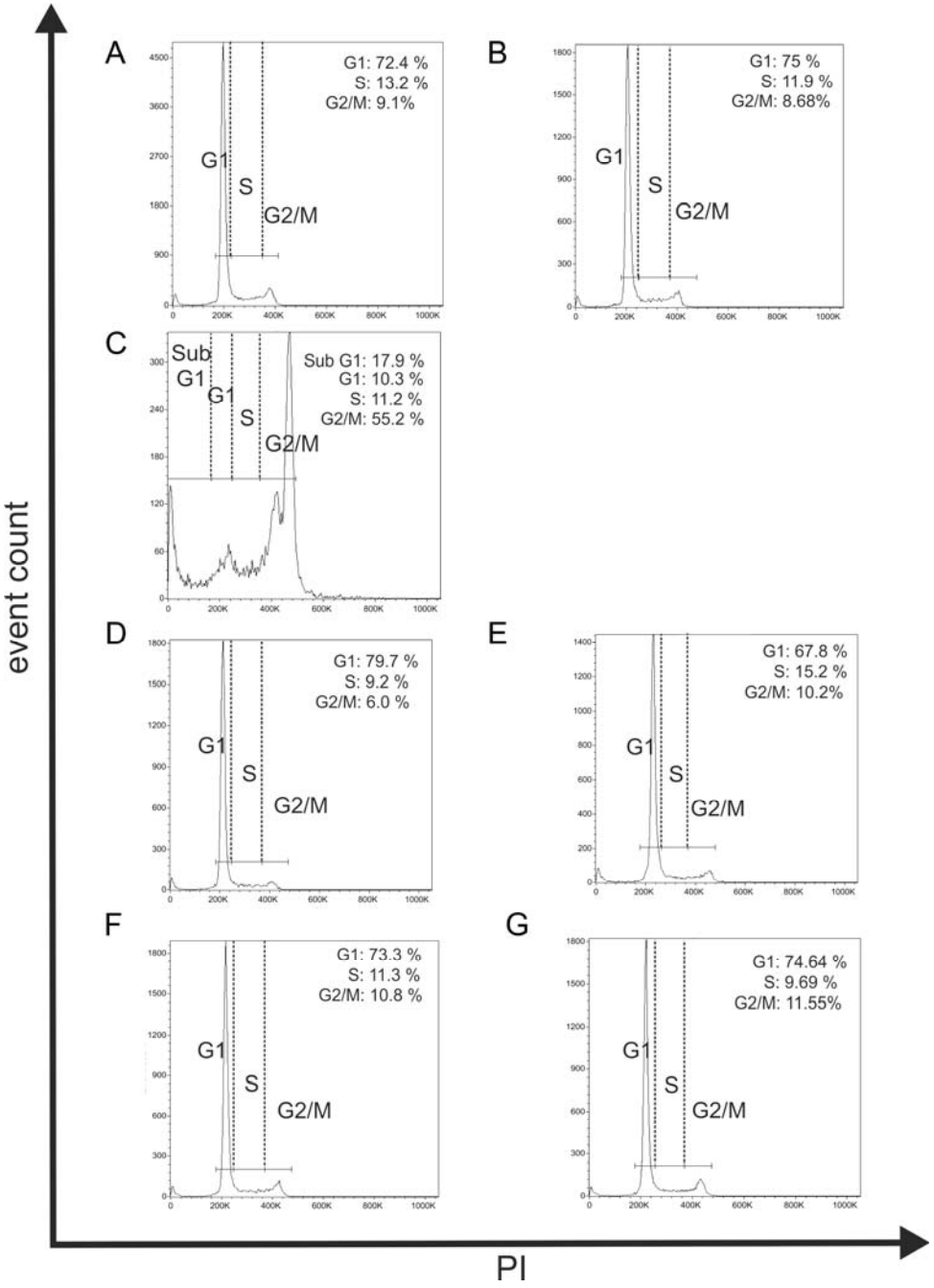

Mass spectra of AGI-B4 (**1**) and methyl (1*R*,2*R*)-1,2,8-trihydroxy-6-(hydroxymethyl)-9-oxo-2,9-dihydro-1*H*-xanthene-1-carboxylate (**2**) obtained using an atmospheric pressure chemical ionization interface. Analyses of the purified compounds by detection of APCI-positive signal fragments and single-quadrupole mass spectrum showing the quasi-molecular ions.

The mass and UV spectra were analyzed with a Hewlett-Packard Series 1100LC-instrument coupled to an AB Sciex 4000 QT mass spectrometer fitted with a Superspher RP18 column (125 x 2 mm, 4 µm particle size, Merck). The chromatographic conditions consisted of a gradient from 1% to 100% acetonitrile in 20 min, and an isocratic step at 100% acetonitrile for 1 min at $40^0$C and 10 µl injection volume was used. The flow rate was 0.45 ml/min. The fragmentor voltage was set to 90 V, the collision energy was set to 10 V in the positive APCI mode and the evaporator

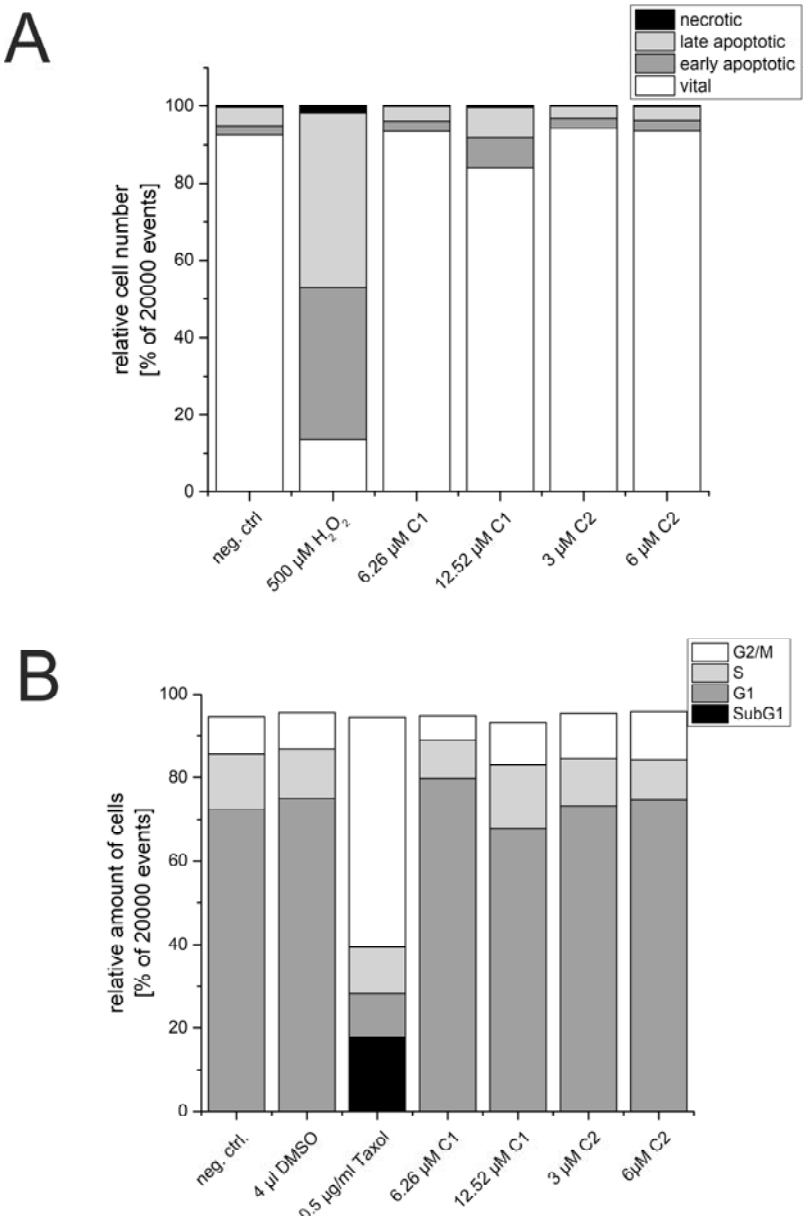
temperature was set to 400°C. The purity of the isolated compounds was analyzed by HPLC-DAD/MS using the conditions described above. The purity of the compounds was greater than 98.5% as determined by HPLC equipped with diode-array detection and mass spectrometry analysis.




Effect of compound **1**, **2** and $H_2O_2$ on induction of necrosis and apoptosis in MonoMac6 cells. 2 x $10^6$ cells were left untreated (**A**), incubated for 2 hours with 500 µM $H_2O_2$ (**B**) or incubated with various concentrations auf compound **1** and **2** for 24 hours. **C:** 6.26 µM **1**; **D:** 12.52 µM **1**; **E:** 3 µM **2**; **F:** 6 µM **2**. Analysis of viable cells was done using Annexin V-Cy5/PI staining by flow cytometry as described in Materials and methods. The data shown is representative of 3 independent experiments.




Cell cycle analysis of MonoMac6 cells treated with taxol and different concentrations of compound **1** and **2**. As a control we used untreated cells (**A**) and cells with 4 µl DMSO added (**B**). **C:** 0.5 µg/ml Taxol; **D**: 6.26 µM **1**; **E:** 12.52 µM **1**; **F:** 3 µM **2**; **G:** 6 µM **2.** Analysis was done after RNA digestion with PI staining. The data shown are representative for three independent experiments.




**A:** Annexin V PI staining of MonoMac6 cells. **B:** Cell cycle analysis of MonoMac6 cells after treatment with compound **1** or **2**.