Quantitative genomic PCR supplementary methods

Needle tissue of *PaSTS*1 Line 5, *PaSTS*1 Line 11 and the vector control was ground to a fine powder under liquid nitrogen. Genomic DNA was isolated from the tissue powder using the method developed by Wright *et al*. (2010) and diluted to a concentration of 100 ng μl^{-1}. PCR was performed with Brilliant SYBR Green QPCR Master Mix ® (Stratagene), 100 ng genomic DNA and 10 pmol forward and 10 pmol reverse primer. *PaSTS* was amplified using the forward primer 5'-CTCGAGATGTCTTCCTCATCTCGTCC-3' and the reverse primer 5'-CCTTCCGTCAGTTCAAAATCTCCGAC -3', designed to amplify 190 base pairs from both *STS*1 and *STS*2 simultaneously. PCR thermocycles were run as follows: 15 min at 95°C followed by 40 cycles of 45 s at 95°C, 30 s at 60°C and 30 s at 72°C using a Stratagene MX3000P thermocycler. *STS* gene abundance was normalized to the abundance of the ubiquitin gene (Schmidt et al., 2010) (GenBank accession number EF681766) amplified with the forward primer 5'-CCCTCGAGGTAGAGTCATCG-3' and the reverse primer 5'-CCAGAGTTCTCCCTTTACTTG-3'. Primer efficiencies were calculated according to Pfaffl (2001). Primer specificity was confirmed by melting curve analysis from 55°C to 95°C and by cloning and sequencing 20 amplicons for each primer pair. Relative *STS* gene copy number in transgenic *PaSTS*1 over-expressing lines was calibrated against the vector control. Each gene abundance represents the rounded average of four biological replicates, each of which is represented by three technical replicates.

Table S1: Forward and reverse attB primers for amplifying and cloning *PaSTS* and *PaCHS* into pDONR207. attB forward and reverse sequences are attached to the 5'end of the sequence specific primer regions

	Forward Primer	**Reverse primer**
attB sequence	5'-GGGGACAAGTTTGTACAAAAAAGCAGGCT-	5'-GGGGACCACTTTGTACAAGAAAGCTGGGT-
STS 1	attBF-CAATGTCTGCAGGAATGACTGTTG-3'	attBR-ATTATGGAAGGAGAACGCTCTTAAGAAC-3'
STS 2	attBF-GAATGACTGTTGATTTGGAAAC-3'	attBR-ATTATGGAAGGAGAACGCTCTTAAGAAC-3'
CHS 1	attBF-GCATGTCTCAGAATTTGGGTTTG-3'	attBR-ATCACTGCAGGGGAACGCTCTTGAG-3'
CHS 2	attBF-GCATGTCTCAGAATTTGGGTTTG-3'	attBR-TTCAATCAGTGCAGGGAACGCTCCTG-3'
CHS 3	attBF-TTATGGCTGGAGGACTGATGGCG-3'	attBR-ATCACTGCAGGGGAACACTCTTCAG-3'
CHS 4	attBF-TTATGGCTGGAGGACTGATGGCG-3'	attBR-ATCACTGCATGGGAACGCTTTTCAG-3'
CHS 5	attBF-TTATGGCTGGAGGACTGATGGCG-3'	attBR-ATTATTGCAGGGGAACGCTCTTGAG-3'
CHS 6	attBF-TTATGGCTGGAGGAATCATGGAG-3'	attBR-ATTATTGCAGGGGAACGCTCTTGAG-3'
CHS 7	attBF-ATATGCCTGCTGGAATGAAGGTGG-3'	attBR-CCTATTGCAGAGGGACGCTCTTGAGAAC-3'
CHS 8	attBF-GCATGCCTGGGACTTTGGGTTTGG-3'	attBR-GTTATTGCGGGCAGGGGACGCTCTTG-3'
CHS 9	attBF-GAATGATGAAGGATCTGGAGG-3'	attBR-ATCACTGCAGGGGAACACTCTTC-3'

Table S2: NCBI accession numbers of CHS and STS sequences used for phylogenetic analysis.

Organism	*Gene*	NCBI accession number	Reference
Vitis vinifera	*CHS*	CAA53583.1	Sparvoli et al., 1994
Vitis vinifera	*RVS 1*	P28343.2	Melchior and Kindl, 1990
Vitis vinifera	*RVS 2*	P51071.1	Melchior and Kindl, 1991
Arachis hypogaea	*CHS*	AAU43217.1	Condori et al., 2009
Arachis hypogaea	*RVS 1*	1Z1EA	Shomura et al., 2005
Arachis hypogaea	*RVS 2*	1Z1FA	Shomura et al., 2005
Arachis hypogaea	*RVS 3*	P51069.1	Lanz et al., 1991
Medicago sativa	*CHS*	AAA02824.1	Junghans et al., 1993
Pisum sativum	*CHS*	CAA44935.1	Ichinose et al., 1992
Trifolium subterraneum	*CHS*	AAA1876.1	Arioli et al., 1994
Glycine max	*CHS*	AAB01004.1	Akada and Dube, 1995
Pyrus communis	*CHS*	AAX16494.1	Fischer et al., 2007
Malus X *domestica*	*CHS*	AAX16492.1	Fischer et al., 2007
Ginkgo biloba	*CHS*	AAS21057	Pang et al., 2004
Pinus densiflora	*STS*	BAA94593.1	Kodan et al., 2002
Pinus densiflora	*STS*	BAA89667.1	Kodan et al., 2002
Pinus strobus	*STS*	CAA87013.1	Raiber et al., 1995
Pinus strobus	*STS*	CAA87012.1	Raiber et al., 1995
Picea sitchensis	*STS 1*	JN400059	
Picea sitchensis	*STS 2*	JN400058	
Picea glauca	*STS 1*	JN400069	
Picea glauca	*STS 2*	JN400070	
Picea abies	*STS 1*	JN400048	
Picea abies	*STS 2*	JN400047	
Picea sitchensis	*CHS 1*	JN400061	
Picea sitchensis	*CHS 2*	JN400062	
Picea sitchensis	*CHS 3*	JN400063	
Picea sitchensis	*CHS 4*	JN400064	
Picea sitchensis	*CHS 5*	JN400065	
Picea sitchensis	*CHS 6*	JN400066	
Picea sitchensis	*CHS 7*	JN400067	
Picea sitchensis	*CHS 8*	JN400068	
Pinus sylvestris	*CHS*	CAA43166.1	Fliegmann et al., 1992
Picea glauca	*CHS 1*	JN400072	
Picea glauca	*CHS 2*	JN400073	
Picea glauca	*CHS 3*	JN400074	
Picea glauca	*CHS 4*	JN400075	
Picea glauca	*CHS 5*	JN400076	
Picea glauca	*CHS 6*	JN400077	
Picea glauca	*CHS 8*	JN400078	
Picea abies	*CHS 1*	JN400050	
Picea abies	*CHS 2*	JN400051	
Picea abies	*CHS 3*	JN400052	

Picea abies	*CHS 4*	JN400053
Picea abies	*CHS 5*	JN400054
Picea abies	*CHS 6*	JN400055
Picea abies	*CHS 7*	JN400056
Picea abies	*CHS 8*	JN400057

Table S3: Forward and reverse primers for quantitative real-time PCR

	Forward Primer	**Reverse primer**
PaUBI	5'-GTTGATTTTTGCTGGCAAGC-3'	5'-CACCTCTCAGACGAAGTAC-3'
***PaLAR*1**	5'-GAACTGGCAGCCATATGGGAGACC-3'	5'-CTGTAATAAAGTTCAGAGGCCTCG-3'
***PaLAR*2**	5'-ACAAGAACTTTTGCATTTAGCCG-3'	5'-GAAATCTCTGGATATAGTTGTGAC-3'
***PaLAR*3**	5'-GGGCATCACGATCTAGAGGTCTG-3'	5'-GGATGGTAAATAGAGGAAGACGAGTC-3'
PaPAL	5'-GTACTTCAGTAGGAGCAGCACTGG-3'	5'-GACATACTCCATGATCGCTGCGG-3'

Table S4: Analytical data of stilbenes and derailment products reported in this manuscript and the basis for compound identification. MS/MS fragments used for compound identification are in bold type.

Experiment title:	Biosynthesis of the major tetrahydroxystilbenes in spruce, astringin and isorhapontin, proceeds via resveratrol and is enhanced by fungal infection
Organism/Plant species:	*Picea*
Organ/tissue:	Bark / Cell free enzyme extracts fed with CoA esters
Analytical tool:	Brucker Daltronics Esquire 6000 ESI ion-trap mass spectrometer

Metabolite	[a)] Ret. Time	Metabolite Class	Mol formula	ES(-) Theor m/z	ES(-) Found m/z	m/z error (Da)	MS/MS ES(-) fragments	MS/MS/MS ES(-) fragments	Principal basis for identification	Maximum UV absorbance	Species from which previously detected	References Reporting spectral data	References reporting spectral data
Astringin (10)	11.2 12.0	Stilbene glycoside	$C_{20}H_{22}O_9$	405.1264	404.9	0.226	**242.7**, 321.7, 200.7	242.7: 240.7, 224.7, 200.7, 184.7, 158.7, 140.8	NMR	331	*Picea sitchensis*		Underwood and Pearce, 1991
Isorhapontin (11)	12.2 12.7	Stilbene glycoside	$C_{21}H_{24}O_9$	419.142	418.9	0.226	**256.7**, 335.7, 214.8	256.7: 215.7, 182.8, 172.7, 157.6, 142.7	MS	331	*Picea sitchensis*		Underwood and Pearce, 1991
Resveratrol (6)	14.6 15.2	Stilbene	$C_{14}H_{12}O_3$	227.0786	226.7	0.379	**184.6**, 164.8, 156.7, **142.7**	184.7: 156.5, 142.7, 116.6,	Commercial standard: Merck (554325-25MG)	331	*Vitis vinifera*	Buiarelli et al., 2007; Lo et al., 2007	Buiarelli et al., 2007; Lo et al., 2007
Piceatannol (7)	13.5 14.1	Stilbene	$C_{14}H_{12}O_4$	243.0736	242.7	0.374	224.7, **200.7**, 174.7, **158.7**, 131.6	200.7: 184.7, 174.6, 158.7, 140.8	Commercial standard:Alexis (ALX-270-202-M001)	331	*Vitis vinifera*	Buiarelli et al., 2007	Buiarelli et al., 2007
Isorhapontigenin (8)	14.6 15.0	Stilbene	$C_{15}H_{14}O_4$	257.0892	256.7	0.389	**214.7**, **172.7**, 158.7	214.7: 172.7, 158.7,	MS		*Picea abies*		Virii et al., 2001

								142.7				
Derailment product (12)	14.2 14.4	Styrylpyrone	$C_{13}H_{10}O_4$	229.0579	228.9	0.158	226.6, **184.7**, 167.7, 158.9, 155.7, **142.7**	184.7: 166.6, 156.7, 152.8, 142.6, 129.7, 112.7	MS	331	Enzyme assay using heterologously expressed resveratrol synthase from *V. vinifera*	Yamaguchi et al,. 1999
Derailment product (13)	13.0 13.5	Styrylpyrone	$C_{13}H_{10}O_5$	245.0528	244.7	0.353	223.3, **200.7**, 172.4, **158.7**	200.7: 186.6, 172.7, 158.6,142.5, 129.8	MS	331	Enzyme assay using heterologously expressed resveratrol synthase from *V. vinifera*	
Derailment product (14)	14.2 14.4	Styrylpyrone	$C_{14}H_{12}O_5$	259.0685	258.9	0.1685	243.7, 226.6, **214.7**, 199.7, **172.7**, 157.8, 148.8	214.8: 199.6, 182.8, 172.7, 157.6, 142.7	MS	331	Enzyme assay using heterologously expressed resveratrol synthase from *V. vinifera*	

[a)] Linear gradient from 0% acetonitrile (100% 0.2% formic acid in water) to 100% acetonitrile in 18 minutes.

Figure S1: Amino acid sequence alignment of STS from *P. abies* (P.a.), *P. sitchensis* (P.s.), *P. glauca* (P.g.) and pinosylvin synthase (PSS) enzymes from *P. densiflora* (P.d.). Dots represent amino acids which are conserved in all presented sequences.

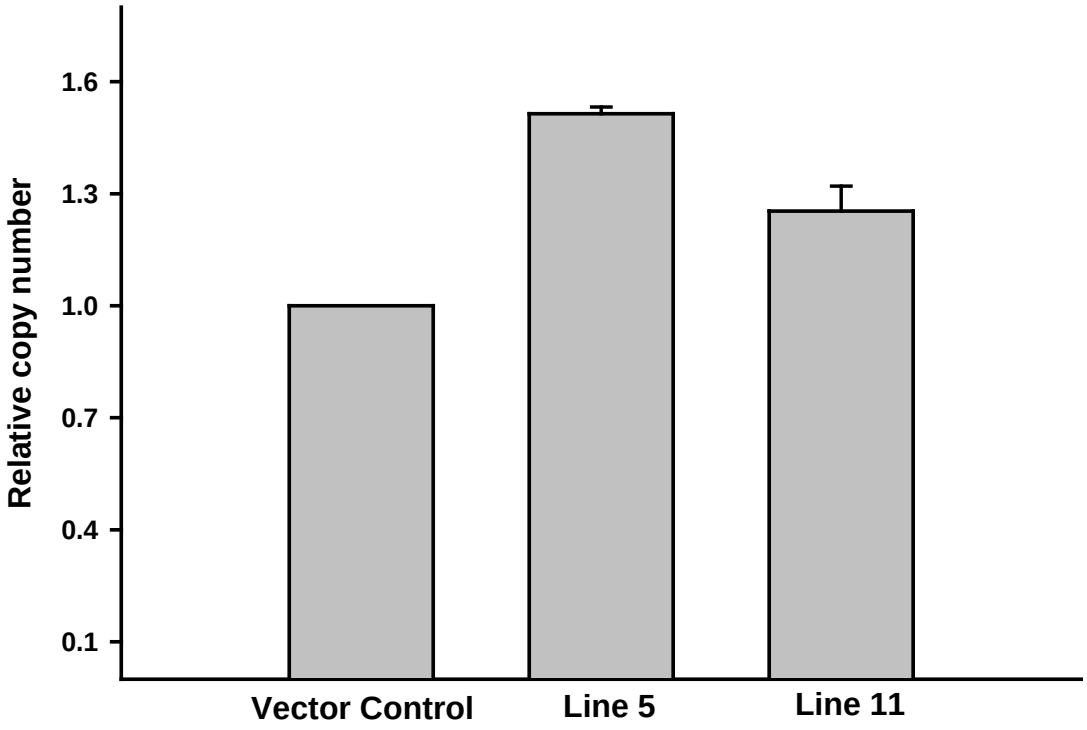

Figure S2: Relative *PaSTS* gene copy number in transgenic *PaSTS*1 lines and vector control.

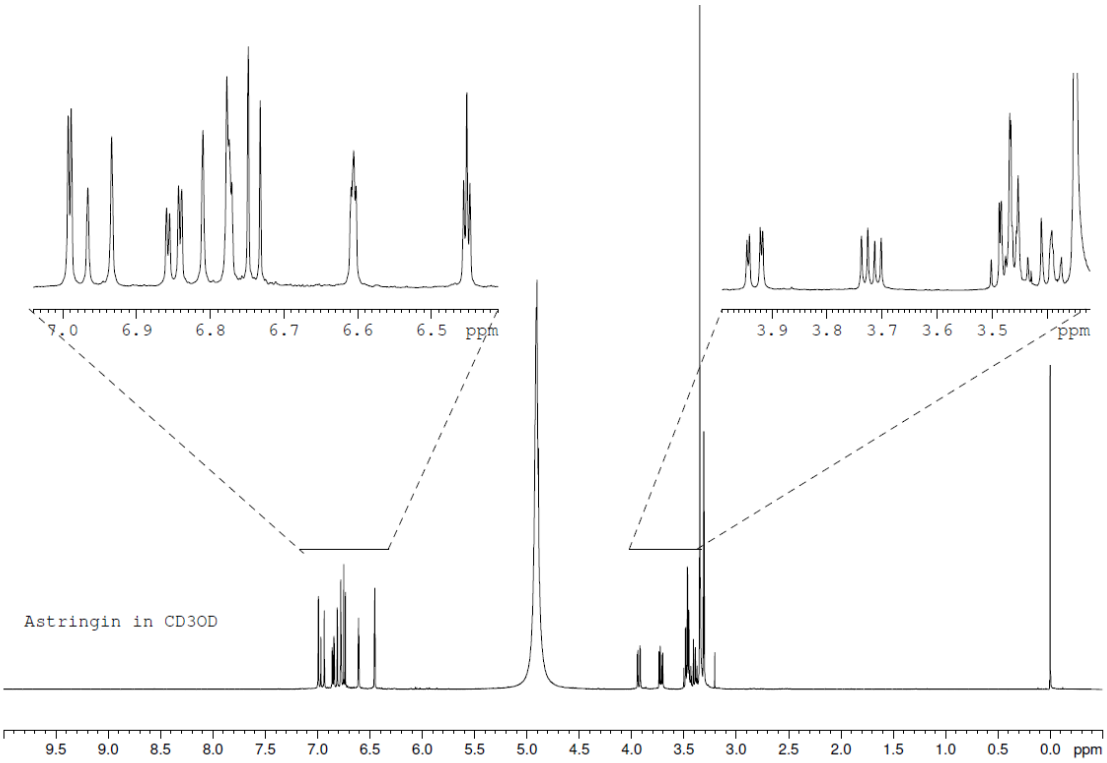

Figure S3: NMR spectrum measured for astringin isolated from *P. abies* in this study was identical to the spectrum described by Li et al. (2008). The signal of the anomeric proton is hidden under the water signal at 4.9 ppm.

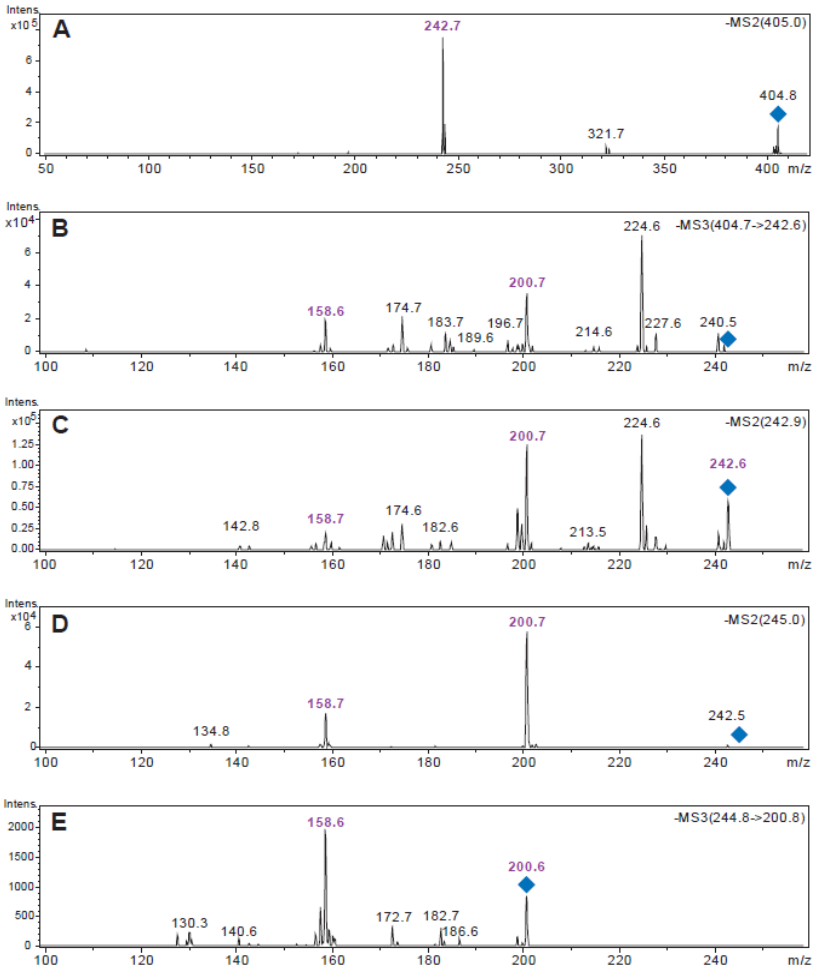

Figure S4: Representative mass fragmentation spectra of tetrahydroxylated stilbene compounds reported in this study as examples of how compound identity was established. Astringin was characterized by its MS/MS spectrum (A) which showed the loss of glucose. In addition an MS^3 spectrum of astringin was obtained (B) in which the fragment 243 (piceatannol – H) was selected for further collision, yielding the diagnostic fragments 201 (M-H) and 159 (M-H), representing the consecutive neutral loss of two ketene moieties. The commercially available piceatannol yielded a similar pattern of MS/MS results (C) as observed in (B). These were used in the characterization of other stilbenes (6, 8) for which no authentic standards were available. The derailment product (E) 6-(3,4-Dihydroxystyryl)-4-hydroxy-2-pyrone, obtained from enzymes assays with caffeoyl CoA as substrate was characterized by its MS/MS (D) and MS^3 (E) spectra yielding the diagnostic fragments 201 (M-H) and 159 (M-H) which theoretically

represent the neutral loss of CO_2 as well as one ketene moiety. These reactions are depicted in Figure S5.

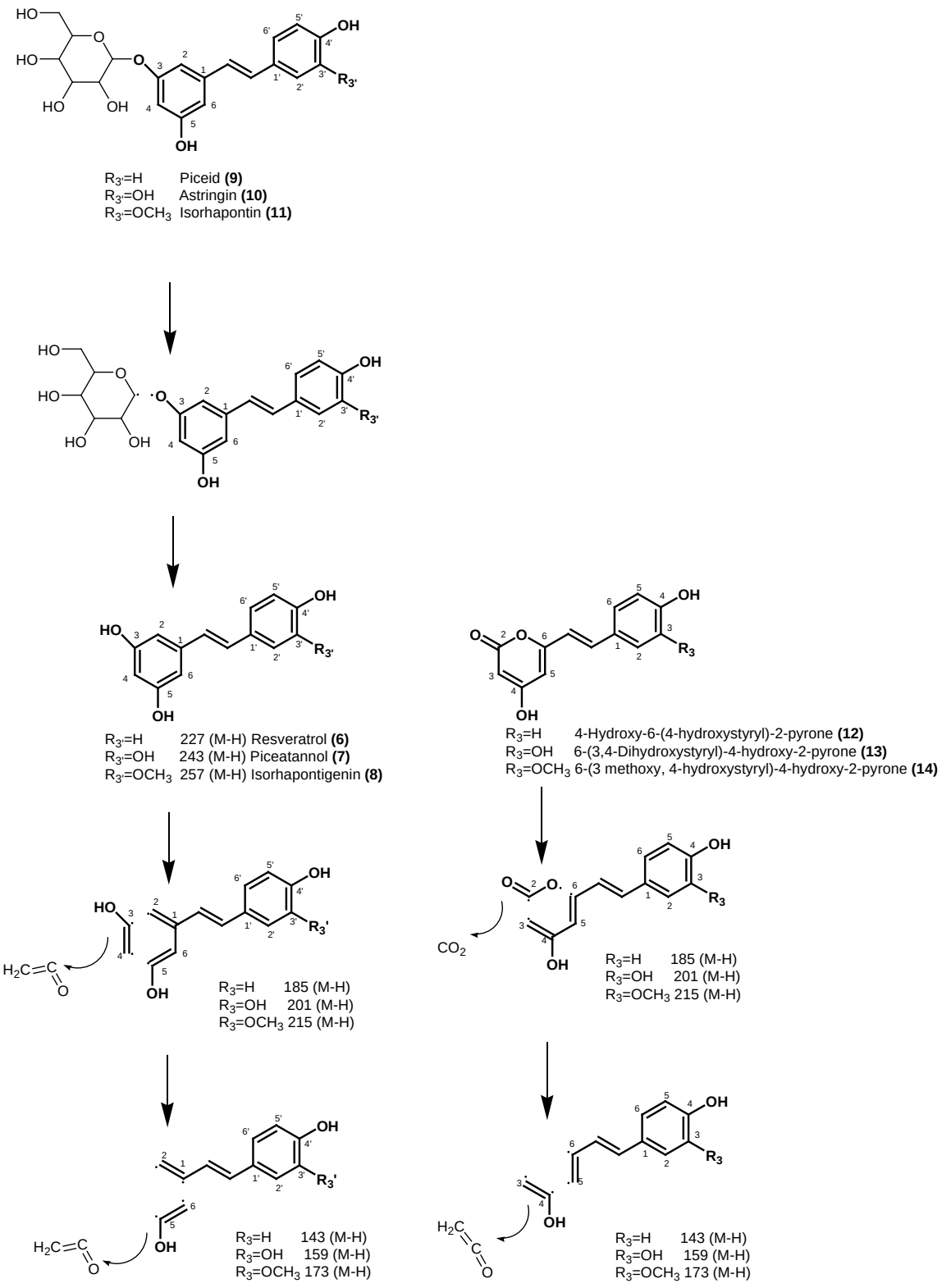

Figure S5: Hypothetical mass fragmentation reactions of stilbenes leading to fragmentation spectra used in identification. During the first fragmentation the glucose moiety is cleaved from the stilbene glycoside, yielding the aglucone. During the second fragmentation of the stilbene, two product ions appear showing neutral losses of 42 and 84 Da, respectively, which have been reported to constitute a sequential loss of two ketene molecules involving positions 3, 4, 5 and 6 on the stilbene skeleton. Fragmentation of styrylpyrone 'derailment products' results in the appearance of two fragments showing neutral losses of 44 and 86 Da, respectively. We hypothesize that similar fragmentation patterns occur in the styrylpyrones as in the stilbenes, resulting in the sequential loss of carbon dioxide and a ketene molecule involving positions 2, 3 and 4 on the pyrone ring.