Stability and reactivity of guaiacylglycerol-β-guaiacyl ether, a compound modeling *β–O–4* linkage in lignin

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1 Structures and mass spectra of Gβ2 and its identified breakdown products

The structures and mass spectra of the compounds used or observed in this study are provided in Figures S1 and S2.

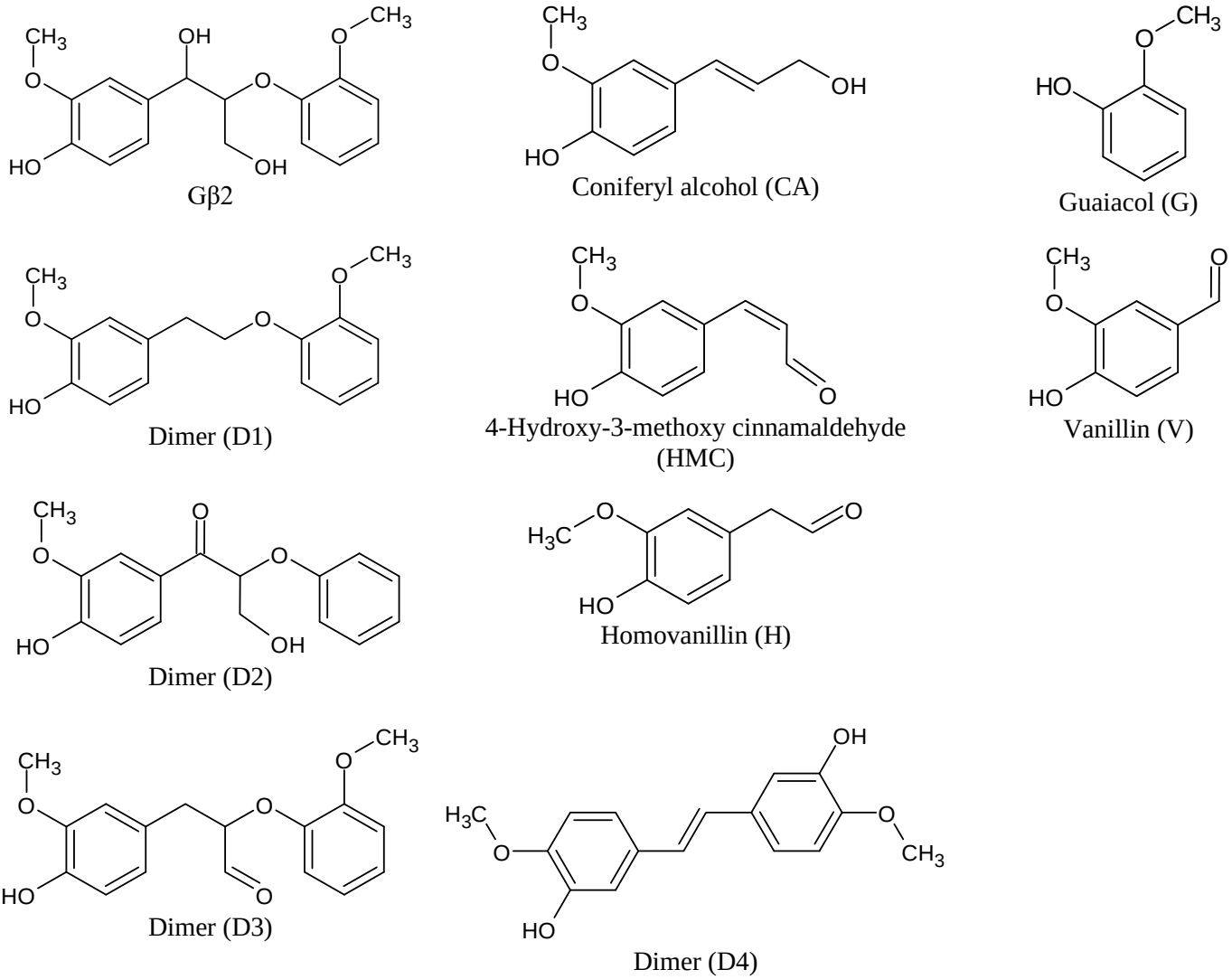
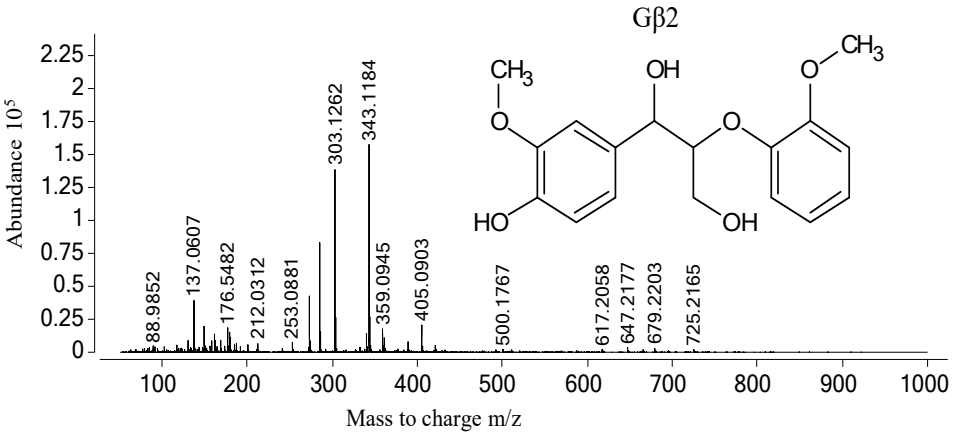
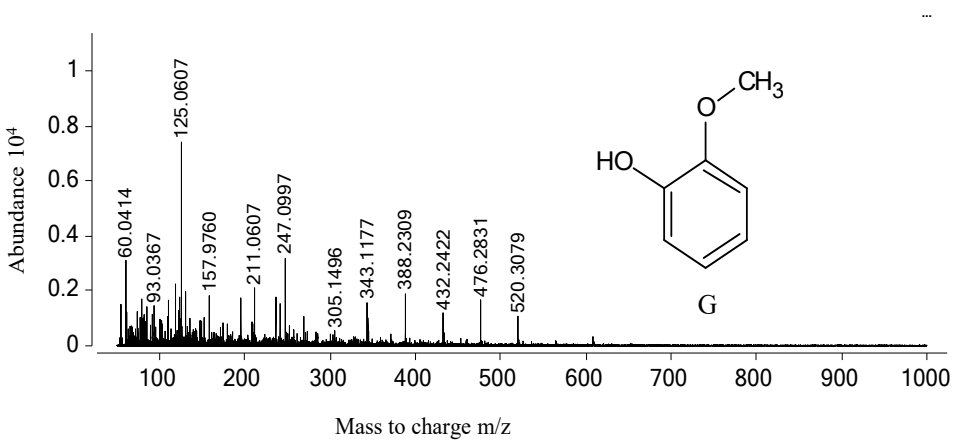


Figure S1. Structures of the compounds considered in this study.


Gβ2
Abundance 10^5
Mass to charge m/z
88.9852
137.0607
176.5482
212.0312
253.0881
303.1262
343.1184
359.0945
405.0903
500.1767
617.2058
647.2177
679.2203
725.2165


Abundance 10^4
Mass to charge m/z
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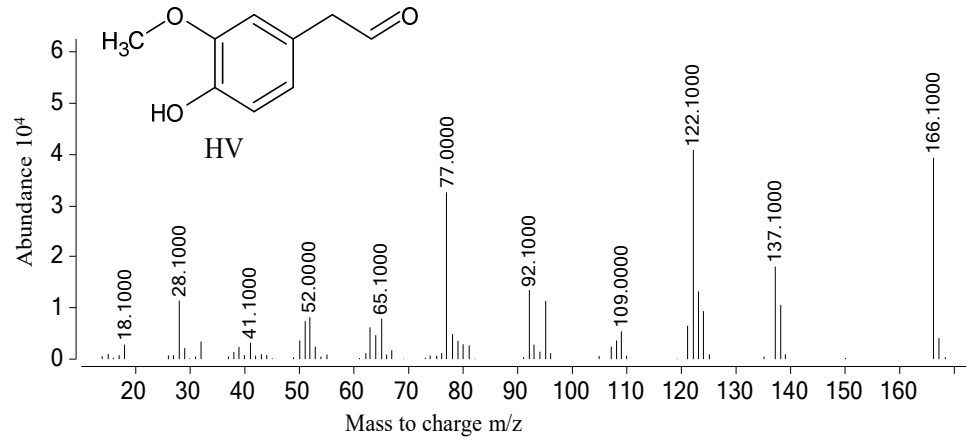
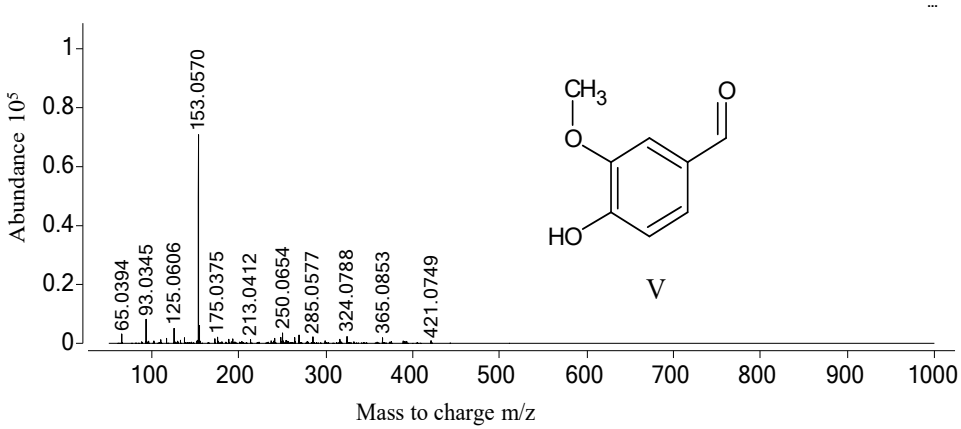
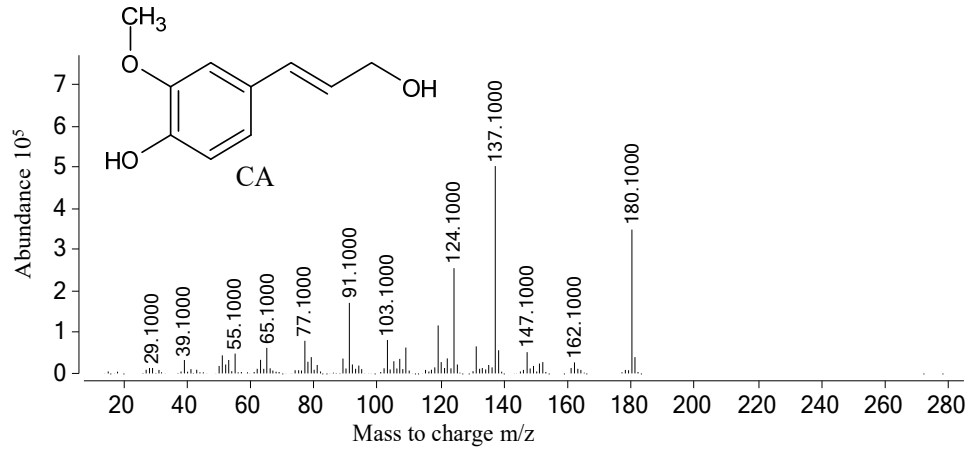
93.0367
125.0607
157.9760
211.0607
247.0997
305.1496
343.1177
388.2309
432.2422
476.2831
520.3079
G


HV
Abundance 10^4
Mass to charge m/z
18.1000
28.1000
41.1000
52.0000
65.1000
77.0000
92.1000
109.0000
122.1000
137.1000
166.1000


V
Abundance 10^5
Mass to charge m/z
65.0394
93.0345
125.0606
153.0570
175.0375
213.0412
250.0654
285.0577
324.0788
365.0853
421.0749


CA
Abundance 10^5
Mass to charge m/z
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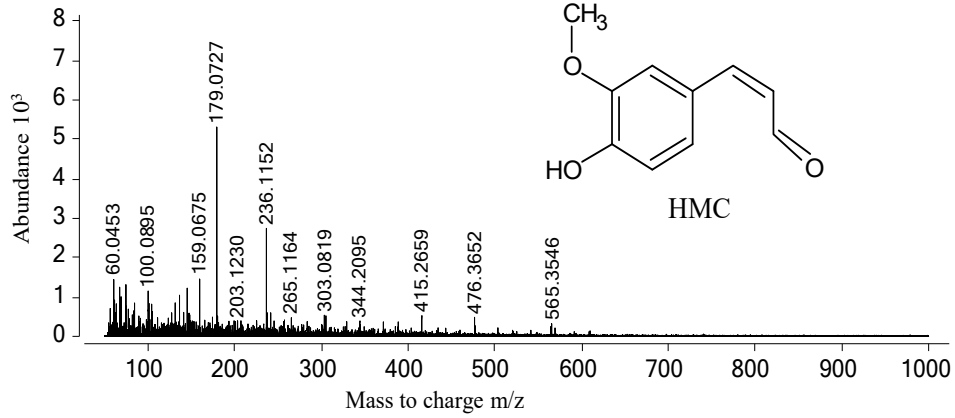
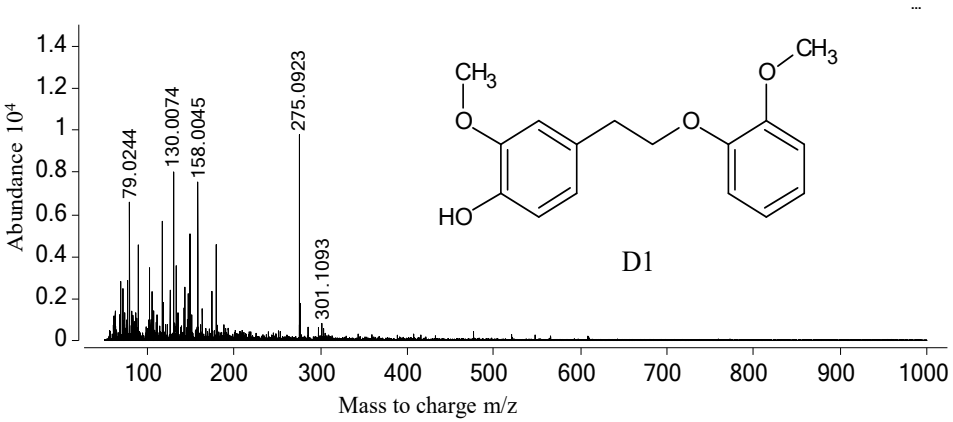
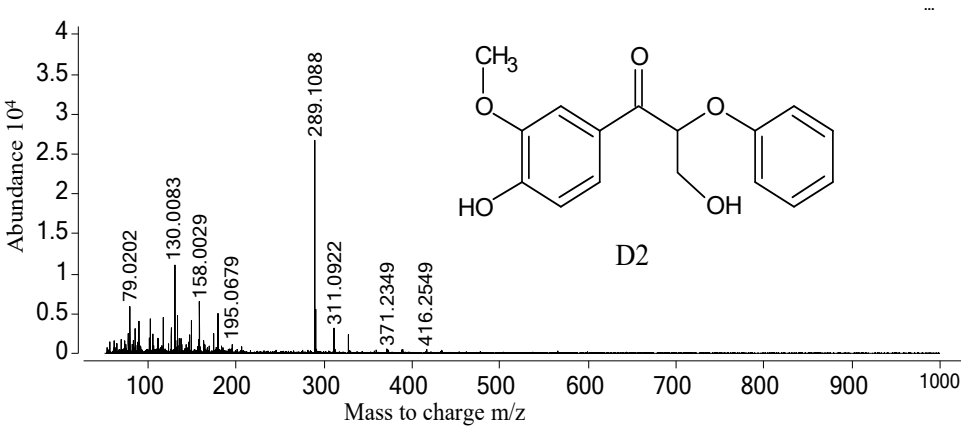
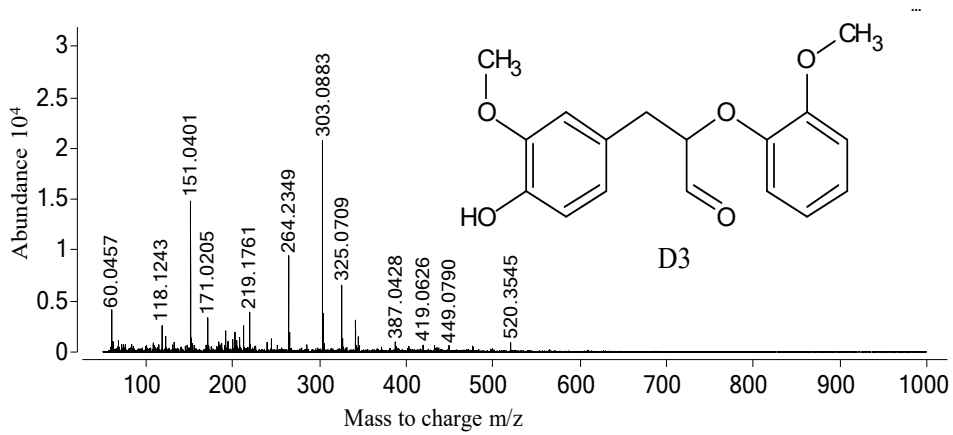
39.1000
55.1000
65.1000
77.1000
91.1000
103.1000
124.1000
137.1000
147.1000
162.1000
180.1000


HMC
Abundance 10^3
Mass to charge m/z
60.0453
100.0895
159.0675
179.0727
203.1230
236.1152
265.1164
303.0819
344.2095
415.2659
476.3652
565.3546







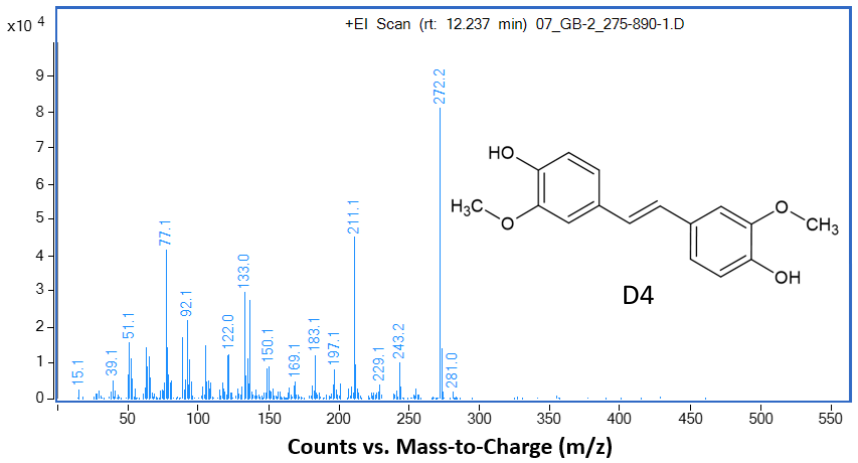


Figure S2. ESI-mass spectra of the compounds observed in this study. MS spectrum for D4 was obtained using GC-EI-MS.

2 Experimental details of the subcritical water experiments

A stock solution of Gβ2 with a concentration of 10,000 μg/mL was prepared. A set of five reaction vessels was prepared containing 0.070 mL Gβ2 and 2.430 mL of water to reach the final concentration, 300 μg/mL. The Gβ2 amount and the water volume were calculated with regards to obtaining subcritical conditions in the given vessel having a length of 6.4 cm and an internal diameter of 0.7 cm. The vessels were sealed with two stainless steel caps of 0.5-1.0 mL volume. The volume of the vessel without the caps was 2.46 mL resulting in the final volume of the sealed vessel being 3.5-4 mL. The presence of the caps ensured a sufficient headspace that was important to maintain the gas/liquid equilibrium, which was crucial for maintaining subcritical conditions and avoiding any potential safety issues resulting from the excessive pressure in the vessel. The procedure of how to calculate the pressure and the portion of the liquid phase inside the vessel in order to ensure safe conditions is as follows:

Determination of cap volume through mass substitution.

1. Mass of H_2O to fill the cylinder and one cap = 3.21 g
2. Density of H_2O at 20.4 °C = 0.9981 g/mL
3. Volume of H_2O in cylinder w/cap = 3.2 mL
4. Cylinder volume = $\pi \times 6.4$ cm $\times (0.70/2 \text{ cm})^2$ = 2.5 cm^3 (mL)
5. Volume of cap = (3) – (4) = 0.77 mL
6. Vessel volume = (4) + 2 × (5) = 3.9 mL

Consequently, saturation conditions of the reaction solvent (water) were obtained for the three tested temperatures: 150, 200, and 250 °C. The information is available online on the National Institute of Standards and Technology (NIST) web page, https://webbook.nist.gov/.

Since the reaction mixture is not water by itself, but, rather, water with Gβ2, it was necessary to determine the density of water in the mixture. For the reaction experiments conducted iat the above-listed three different temperatures, 0.07 mL of Gβ2 was used. Assuming that Gβ2 is not

compressible, the potential non-additivity of volumes can be ignored due to the small amounts of Gβ2. Therefore as water fills the remainder of the internal vessel space, the density of water in the mixture with Gβ2 can be calculated the following way:

$$\text{Density of } H_2O = \frac{\text{Mass of water}}{V_{vessel} - V_{G\beta2}} = \frac{2.4\ \text{g}}{3.983\ \text{mL} - 0.07\ \text{mL}} = 0.61\ \text{g/mL}$$

3 Optimization of ESI conditions in subcritical water treatment experiments

Capillary and fragmentor voltages were evaluated in terms of ionization efficiency. Table S1 presents the summary of experimental conditions used for direct infusion analysis of Gβ2 with ESI in positive and negative modes with different solvents. The selection of solvent and ionization mode (positive or negative) had a clear impact on their ionization. As a result of these experiments, formic acid and 50% acetonitrile appear to be the most suitable electrolyte and solvent, respectively.

Table S1: Acquired mass responses for target ions dissolved in various solvent and electrolyte systems in positive/negative mode and varying fragmentor and capillary voltages performed using direct infusion.

	Capillary/Fragmentor voltages (drying gas: 350 °C and 12 L/min; nebulization pressure 25 psig)									
		3000/120	3000/150	3500/100	3500/120	3500/150	3500/180	4000/150	4500/150	5000/150	5500/150
Gβ2	2.5 mM FA +%50 ACN/pos	1500	1800	1800	2000	2300	2500	2200	2200	2000	
	2.5 mM FA +%50 ACN/neg	60	70	80	113	95	50	80			
	2.5 mM FA +%50 MeOH/pos	1000	1200	1200	1550	1800	1850	2000	2300	2900	3600
	2.5 mM FA +%50 MeOH /neg	55	60	62	70	88	90	105	98		
	2.5 mM NH_4OAc +%50 ACN/pos	-	-	330	385	490	570	650	640	600	440
	2.5 mM NH_4OAc +%50 ACN/neg	30	33	44	51	68	77	80	55		
	2.5 mM NH_4OAc +%50 MeOH /pos	340	510	603	660	684	709	800	1100	1200	1425
	2.5 mM NH_4OAc +%50 MeOH /neg	-	-	49	52	55	60	63	50		

Using direct infusion, the prepared solutions were directly injected to TOF-MS at a flow rate of 10 μL/min. The capillary voltage was optimized between 3000-6000 V in positive mode and 2000-

4000 V in negative mode. The fragmentor voltage was optimized between 110 -180 V. Table S1 represents the best responses for Gβ2.