Supplemental Material

for

Immunomodulatory Activity of Oenothein B Isolated from *Epilobium angustifolium*

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Supplemental Table S1. NMR Spectroscopy of Subfraction S-3

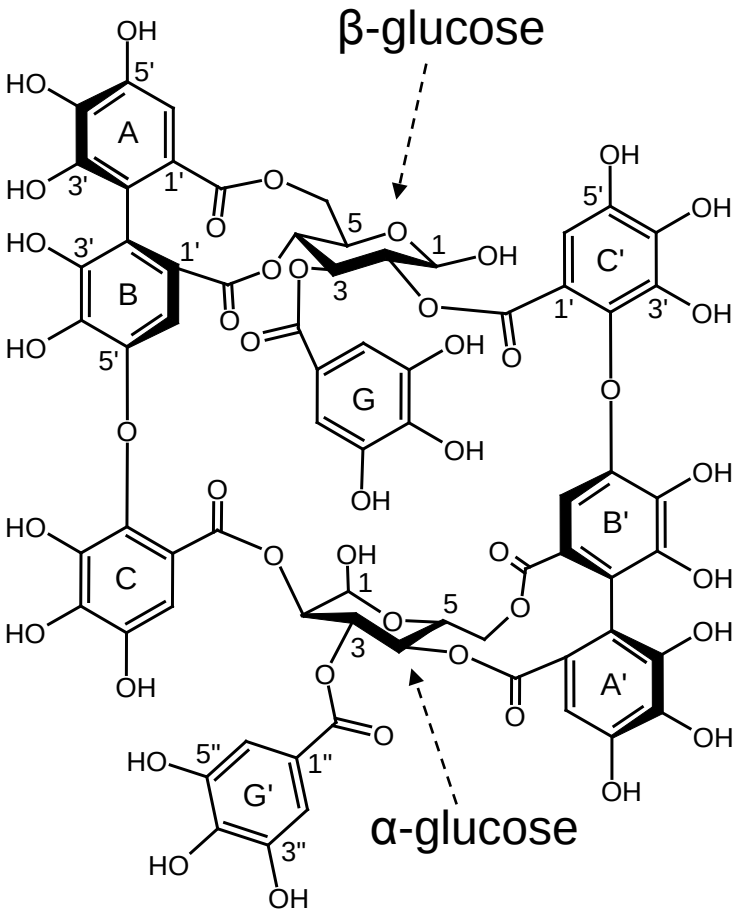
^{1}H NMR [500 MHz, D_2O]

β-glucose: δ 4.47 (*d*, *J* 9 Hz, H-1), 5.09 (*dd*, J_1 6.5, J_2 9 Hz, H-2), 5.29 (*t*, *J* 9.5 Hz, H-3), 4.89 (*t*, *J* 10.5 Hz, H-4), 3.92 (*dd*, J_1 5.5, J_2 10.5 Hz, H-5), 4.45 (*d*, *J* 12.5 Hz, H-6), 3.75 (*d*, *J* 12.5 Hz, H-6); **ring A**: 6.41 (*s*, H-6); **ring B**: 6.28 (*s*, H-6′); **ring C**: 6.53 (*s*, H-6′); **ring G**: 6.95 (2H, *s*, H-2″ and H-6″); **α-glucose**: 5.47 (*d*, *J* 3.4 Hz, H-1), 5.36 (*dd*, J_1 3.4, J_2 10.5 Hz, H-2), 5.60 (*t*, *J* 10 Hz, H-3), 4.99 (*t*, *J* 9 Hz, H-4), 4.53 (*dd*, J_1 7, J_2 9 Hz, H-5), 4.48 (*d*, *J* 12 Hz, H-6), 3.73 (*d*, *J* 12 Hz, H-6); **ring A′**: 6.15 (*s*, H-6′); **ring B′**: 6.15 (*s*, H-6′); **ring C′**: 6.42 (s, H-6′); **ring G′**: 6.57 (2H, *s*, H-2″ and H-6′).

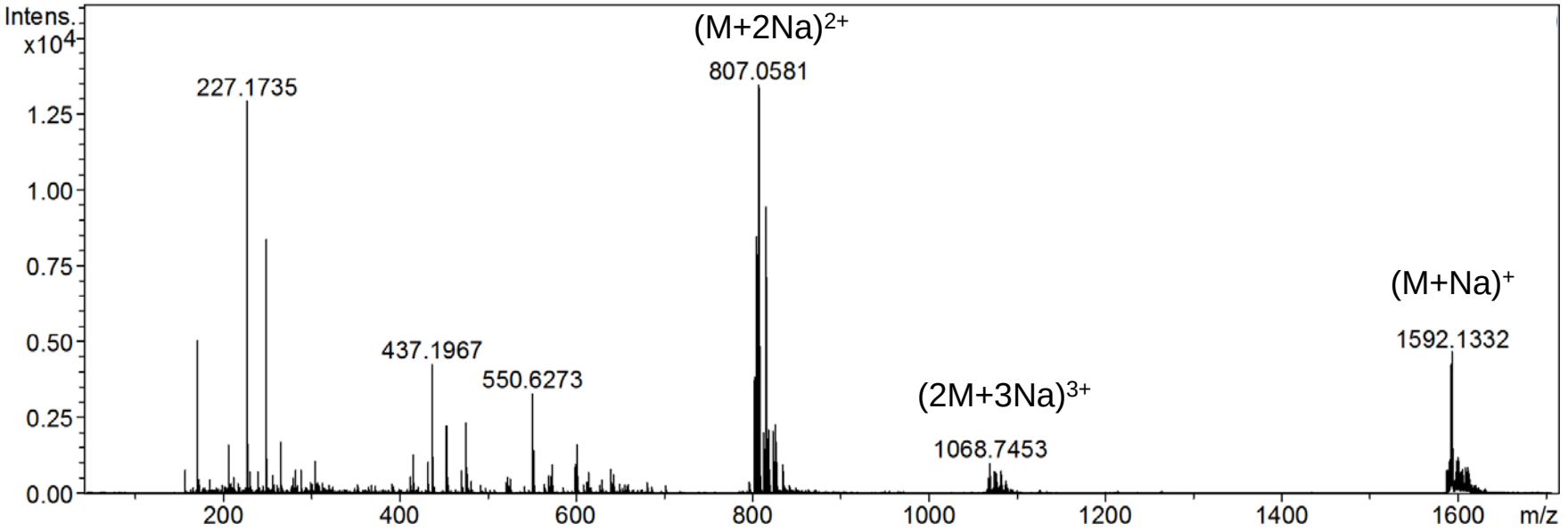
^{13}C NMR [400 MHz, D_2O]

β-glucose: δ 94.8 (C-1), 74.8 (C-2), 71.0 (C-3), 72.2 (C-4), 70.6 (C-5), 63.5 (C-6); **ring A**: 124.6 (C-1′), 116.3 (C-2′), 143.5 (C-3′), 136.1 (C-4′), 145.8 (C-5′), 106.5 (C-6′), 169.8 (CO); **ring B**: 125.0 (C-1′), 116.7 (C-2′), 146.4 (C-3′), 134.4 (C-4′), 148.0 (C-5′), 105.4 (C-6′), 169.5 (CO); **ring C**: 115.0 (C-1′), 139.7 (C-2′), 140.5 (C-3′), 140.8 (C-4′), 142.9 (C-5′), 113.7 (C-6′), 168.8 (CO); **ring G**: 119.2 (C-1″), 108.7 (C-2″ and C-6″), 144.3 (C-3″ and C-5″), 138.6 (C-4″), 166.3 (CO); **α-glucose**: 89.5 (C-1), 74.3 (C-2), 69.9 (C-3), 69.5 (C-4), 67.4 (C-5), 63.2 (C-6); **ring A′**: 121.4 (C-1′), 115.1 (C-2′), 144.0 (C-3′), 134.8 (C-4′), 144.8 (C-5′), 106.9 (C-6′), 169.1 (CO); **ring B′**: 124.8 (C-1′), 116.7 (C-2′), 144.3 (C-3′), 135.9 (C-4′), 147.5 (C-5′), 106.5 (C-6′), 169.6 (CO); **ring C′**: 114.3 (C-1′), 137.5 (C-2′), 139.1 (C-3′), 141.6 (C-4′), 142.3 (C-5′), 109.8 (C-6′), 167.1 (CO); **ring G′**: 117.7 (C-1″), 110.3 (C-2″ and C-6″), 144.4 (C-3″ and C-5″), 138.6 (C-4″), 169.4 (CO).

Supplemental Figure S1. Chemical Structure of Oenothein B

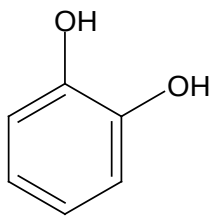


Supplemental Figure S2. Mass Spectrum of Subfraction S-3

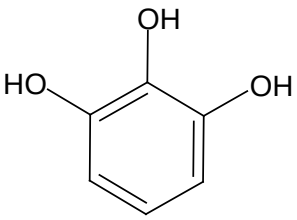


Mass spectrometry experiments were performed using a Bruker Microtof high resolution time of flight mass spectrometer (Bruker Daltonics, Inc., Billerica, MA).

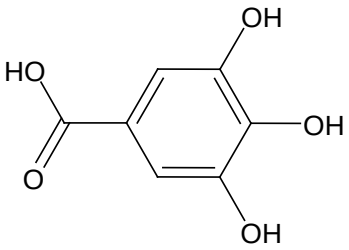
Supplemental Figure S3. Structures of Related Compounds Under Investigation



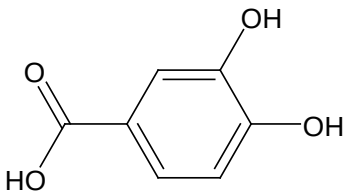
Pyrocatechol



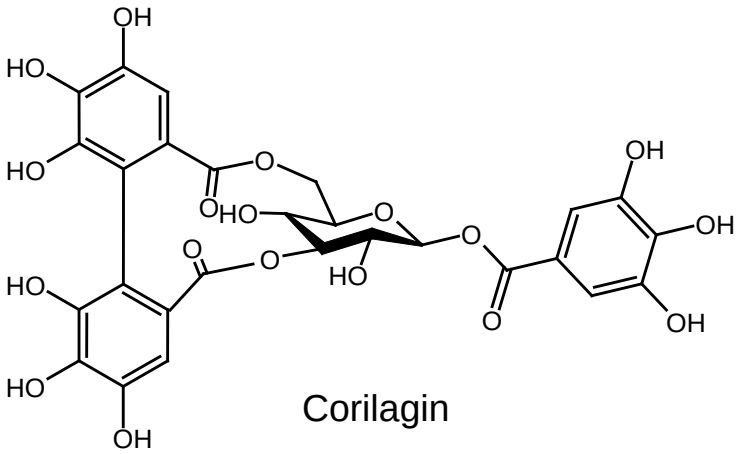
Pyrogallol



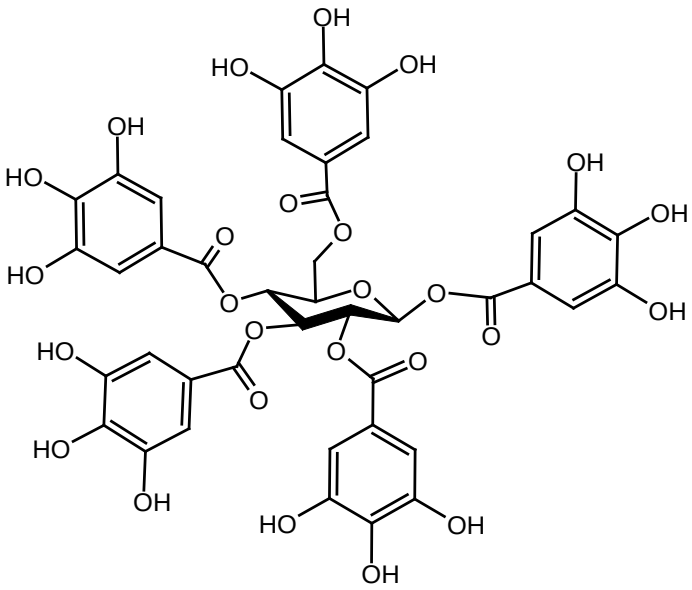
Gallic acid



3,4-dihydroxybenzoic acid (protocatechuic acid)

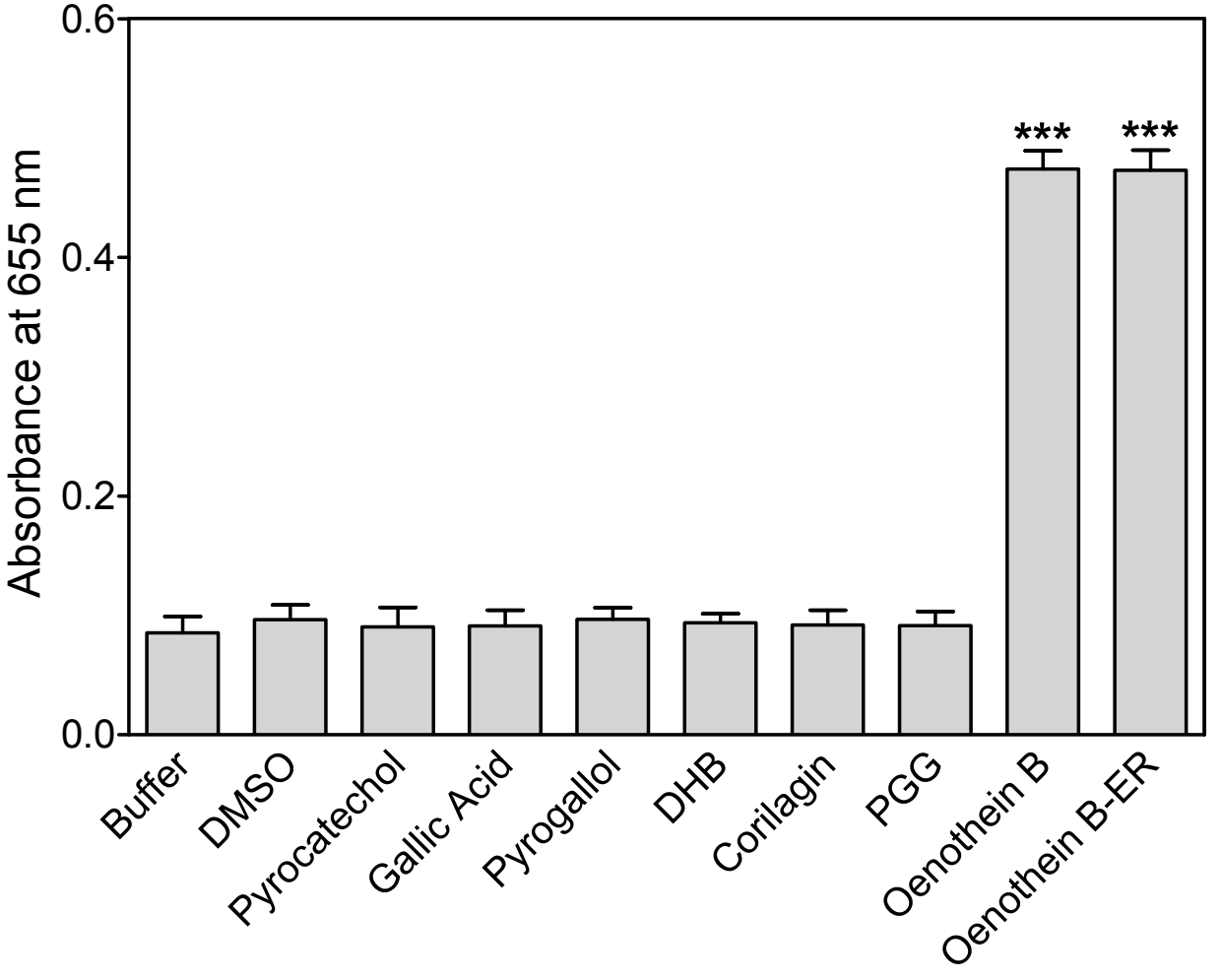


Corilagin



1,2,3,4,6-pentakis-O-galloyl-β-D-glucose (PGG)

Supplemental Figure S4. Effect of Oenothein B and Related Compounds on NF-κB Activity



THP1-Blue monocytes (2×10^5 cells/well) were incubated for 24 hr with 100 μM pyrocatechol, gallic acid, pyrogallol, and 3,4-dihydroxybenzoic acid (DHB); 50 μM corilagin and PGG; and 25 μM oenothein B (OB) and oenothein B pretreated with endotoxin-removing gel (OBr) . Alkaline phosphatase release was analyzed spectrophotometrically in the cell supernatant. The data are presented as mean±S.D. of triplicate samples from one experiment that is representative of three independent experiments. Statistically significant differences (***P<0.001) versus buffer or DMSO controls are indicated.