Supporting Information

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Isolation of Phytochemicals from *Cordia rothii* (Boraginaceae) and Evaluation of their Immunomodulatory Properties

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Experimental details

Chromatographic Material

Organic solvents used were purchased from Merck. Silica gel 60 (70-230 mesh, E. Merck, Damstadt, Germany) was used for column chromatography. *Sephadex LH*-20 (25-100 µm; Sigma-Aldrich) and C-18 (25-40 µm, 10 nm; Macherey-Nagel) were used to perform reverse phase column chromatography. Vacuum liquid chromatography (VLC) was conducted using silica gel 60 HF_{254} (Merck). Recycling Preparative HPLC (Model LC-908W/G10/G30/C60) was used to purify compounds from the highly polar fraction. Purity of compounds was checked on silica gel 60 GF_{254} precoated cards (0.2 mm thickness) and RP-18 F_{254S} aluminum sheets (20x20 cm, Merck, Darmstadt, Germany).

Instrumentation

UV data was taken on Thermo Scientific (Model: Evolution 300) UV-Visible Spectrophotometer. IR was recorded on Bruker Vector 22. EIMS was performed on JEOL, The MS route JMS-600H, HREIMS on Thermo Finnigan MAT 95 XP and FABMS (+ve/-ve) on JEOL JMS-HX110 Mass Spectrometer. ESI-MS was taken on Applied Biosystem Q-STAR XL, in the positive ion mode. The ^{1}H NMR experiments were recorded in $CDCl_3$, C_5D_5N, C_3D_6O and CD_3OD on Bruker Avance 300, 400, 500 and 600 spectrometers working at 300, 400, 500 and 600 MHz respectively, whereas ^{13}C NMR (BB & DEPT) were measured at 75, 100, 125, and 150 MHz. Chemical shifts (δ) were calculated in ppm and coupling constants (*J*) in Hertz (Hz).

Extraction and Isolation

Uncrushed dried leaves (5 Kg) of *C. rothii* were soaked in MeOH (3 x 20 L), each time for 15 days. The extract obtained was filtered and evaporated under reduced pressure to give residue CRM (85.23 g). CRM was treated with water and extracted sequentially into *n*-hexane (CRMH, 57.72 g), EtOAc (CRME, 11.14 g), and BuOH (CRMB, 12.24 g) to yield corresponding phases.

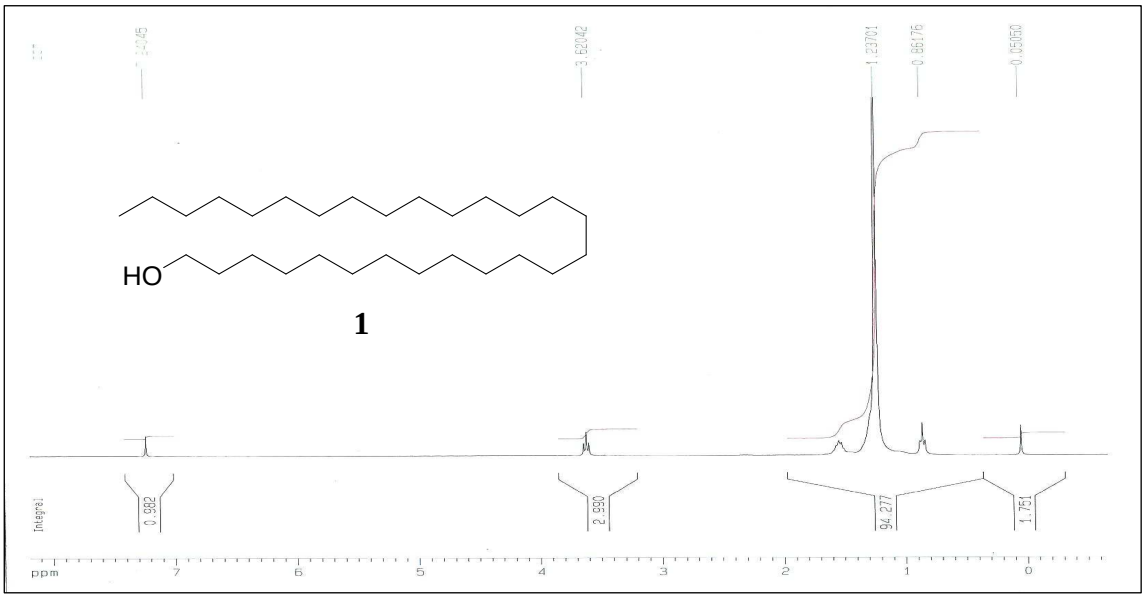
CRMH was partitioned into aqueous methanol and hexane soluble layer which upon evaporation under reduced pressure provided methanol (CRMHM, 14.78 g) and hexane soluble residues (CRMHH, 28.73 g) respectively.

The CRMHH provided different fractions of low polarity when subjected to vacuum liquid chromatography. The sub-fractions were collected from *n*-hexane (BH, 6.90 g), CCl_4 (BCT, 3.16 g), CH_2Cl_2 (BD, 4.25 g), $CHCl_3$ (BCH, 3.41 g), EtOAc (BE, 4.53 g) and $(CH_3)_2CO$ (BA, 3.57 g). From BD, an insoluble solid settled down, which on washing yielded purified compound (**1)** (1.58 mg). BCH provided crystalline compound (**2)** (1.38 mg) through re-crystallization in $CHCl_3$. BE upon column chromatography on silica gel column using isocratic mobile phase $CHCl_3$-MeOH (6.75:3.25 v/v) provided 79 fractions. Of these, fractions 1-15, 16-53, and 54-79 on pooling yielded three subfractions; BE1, BE2, and BE3, respectively. BA upon column chromatography on silica gel with isocratic system $CHCl_3$-MeOH (9:1 v/v) yielded 31 fractions. On standing an insoluble solid separated out from the first three pooled fractions of this column, which upon filtration and washing furnished (**3**) (1.67 mg). The filtrate upon vacuum evaporation yielded a residue, which upon column chromatography on silica gel $CHCl_3$-MeOH (9.25:0.75 v/v) provided 32 fractions. On the

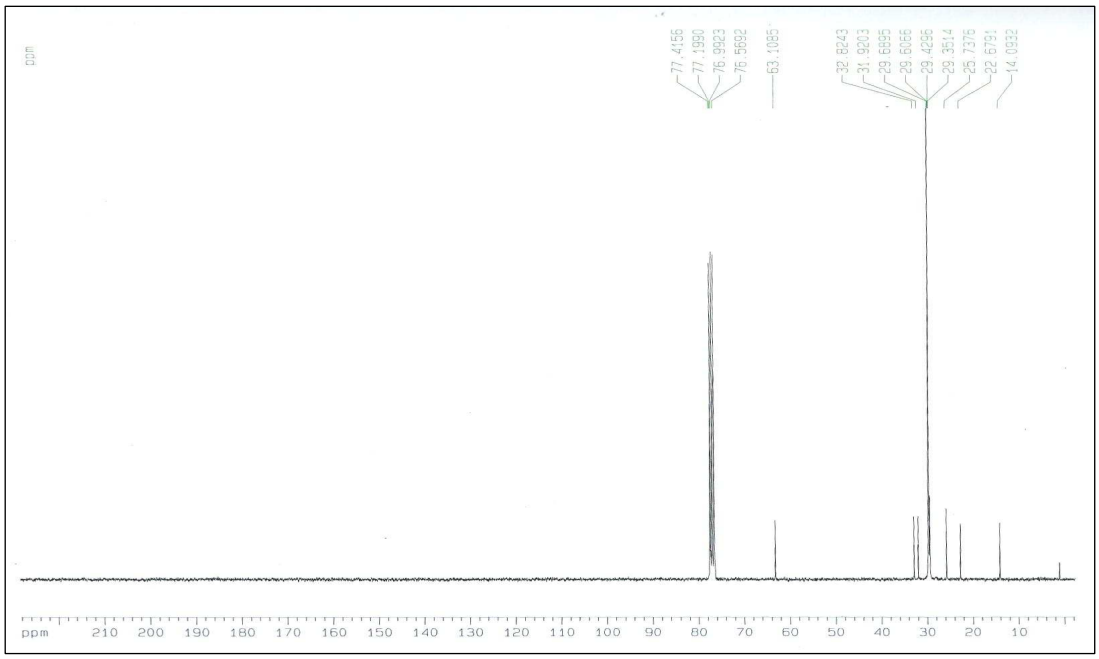
basis of TLC, fractions were differentiated. Three subfractions; BA1, BA2, and BA3 containing Fractions 1-4, 6-8, and 9-12 respectively were selected for further purification on the basis of amounts. **(4)** (5.12 mg) was obtained and purified from the subfraction BA3.

The residue CRME was partitioned into neutral CRMEE (5.46 g) and acidic CRMEA (0.99 g) fractions by treating it with 4% Na_2CO_3 and neutralizing it with 5% HCl solution, respectively. The CRMEE was loaded on silica gel column. Using gradient elution ($CHCl_3$, EtOAc, and MeOH) it afforded 118 fractions; of these, fraction 16-24 (C2) and fraction 36 (C6) were screened for immunomodulatory properties. Fractions 27-29 (C4) upon repeated column chromatography on silica gel $CHCl_3$-MeOH (9.8:0.2 v/v) **(5)** (3.25 mg) and fraction 37-48 (C7) provided another crystalline compound **(6)** (5.89 mg) *via* crystallization. The residue CRMB (12.24 g) upon column chromatography on silica gel, with gradient elution using $CHCl_3$, MeOH, and H_2O yielded 79 fractions. Fraction 29 (D1), fraction 30 (D2), fraction 31 (D3), and fraction 32 (D4) were screened for immunomodulatory properties while collective fractions 33-40 (D5, 2.85 g) upon column chromatography on silica gel with gradient elution using $CHCl_3$ and MeOH provided 68 fractions. Fractions 36-51 upon repeated column chromatography on silica gel CH_2Cl_2-MeOH (9:1 v/v) furnished pure compound **(7)** (4.56 mg). Fraction 52-59 on silica gel column chromatography CH_2Cl_2-MeOH (9:1 v/v) yielded another 91 fractions. Compounds **(8)** (3.15 mg), **(9)** (4.25 mg), and (**10)** (2.35 mg) were obtained and purified from the pooled subfractions 13-29 of this column using recycling preparative HPLC MeOH-H_2O (7:3 v/v).

*1-Octacosanol (**1**)*:White amorphous powder. IR ν_{max} (KBr) cm^{-1}: 3420, 2919, 2850, 1467, 723.^{1}H NMR ($CDCl_3$, 300 MHz) δ_H: 0.86 (3H, t, *J* = 6.0 Hz, H-28), 1.24 (50H, br.s, H-3-H-27), 1.54 (2H, m, H-2), 3.62 (2H, t, *J* = 6.6 Hz, H-1).^{13}C NMR ($CDCl_3$, 75 MHz) δ_C:14.1(CH_3, C-28), 22.7 (CH_2, C-27), 25.7 (CH_2, C-3), 29.7 (CH_2, C-4-C-25), 31.9 (CH_2, C-26), 32.8 (CH_2, C-2).EIMS *m/z* (rel. intensity, %): 392 $[M - H_2O]^+$ (12), 111 (23), 97 (51), 85 (25), 83 (71), 71 (46), 69 (72), 57 (100).HREIMS: *m/z* 410.4040 $[M]^+$ (calcd. for $C_{28}H_{58}O$, 410.44876, Δ 0.04 ppm).CI (+): 409 $[M - H]^+$.



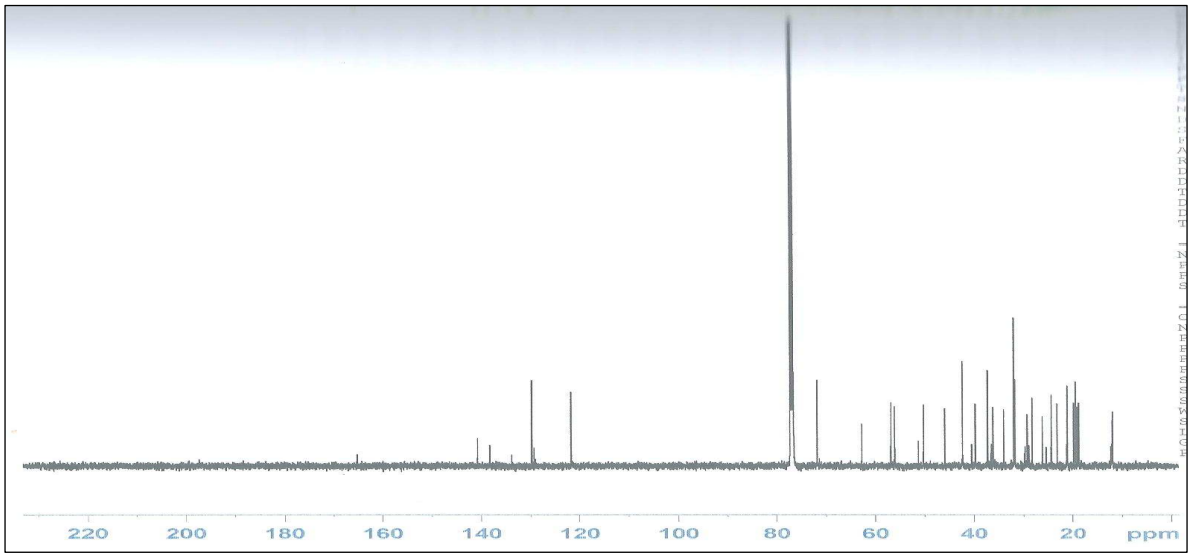
S1: ^{1}H-NMR spectrum of 1-octacosanol (**1**) in $CDCl_3$ (300 MHz)



S2: ^{13}C-NMR spectrum of 1-octacosanol (**1**) in $CDCl_3$ (75 MHz)

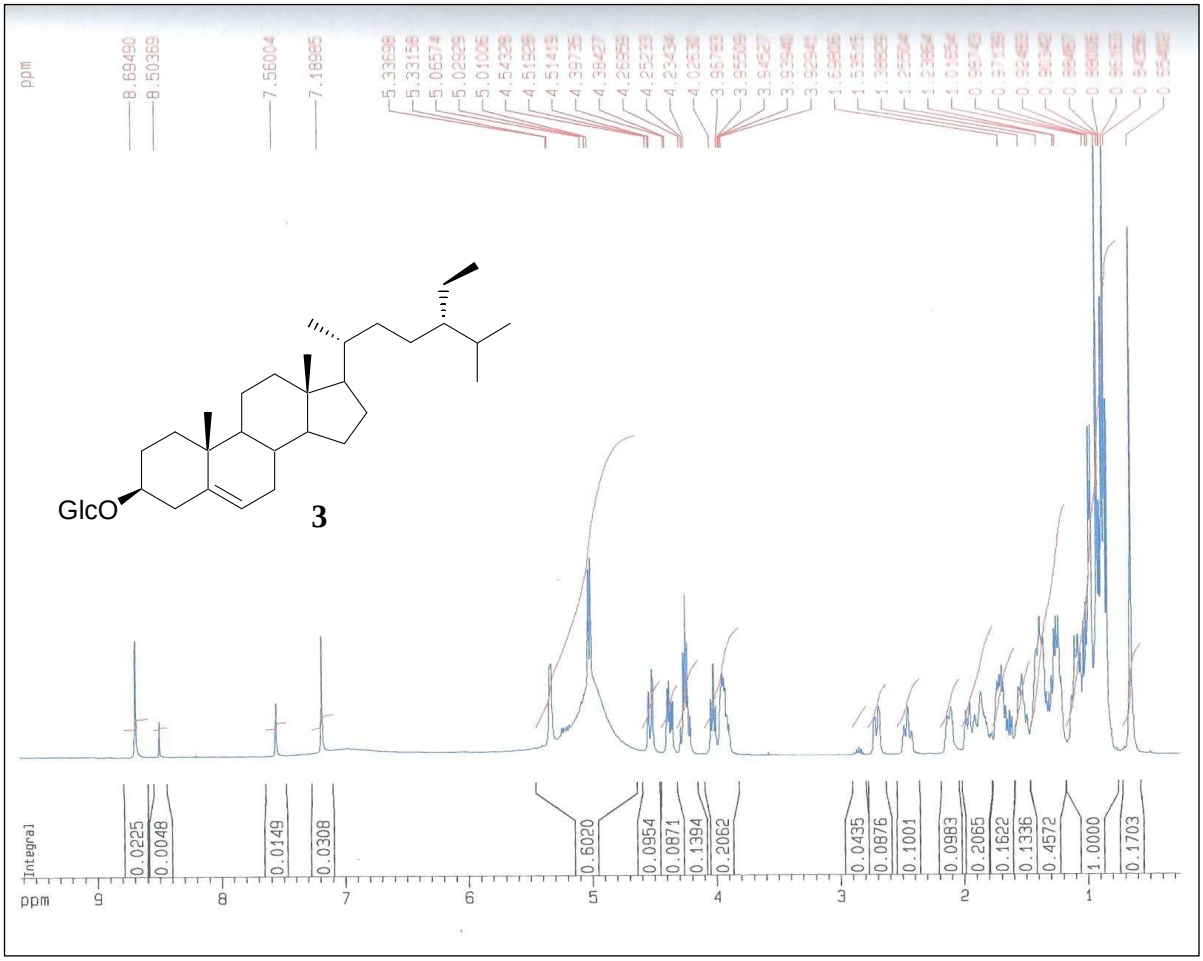
*β-Sitosterol (**2**):*White shiny crystals. ^{1}H NMR ($CDCl_3$, 500 MHz) δ_H: 0.66 (3H, s, H-18), 0.79 (3H, d, *J* = 7.0 Hz, H-27), 0.81 (3H, d, *J* = 6.5 Hz, H-26), 0.83 (3H, t, *J* = 7.0 Hz, H-29), 0.90 (3H, d, *J* = 6.5 Hz, H-21), 0.99 (3H, s, H-19), 3.50 (1H, m, H-3), 5.33 (1H, m, H-6).^{13}C NMR ($CDCl_3$, 125 MHz) δ_C: 11.9 (CH_3, C-29), 12.1 (CH_3, C-18), 18.8 (CH_3, C-21), 19.1 (CH_3, C-27), 19.4 (CH_3, C-19), 19.8 (CH_3, C-26), 21.1 (CH_2, C-11), 23.1 (CH_2, C-28), 24.3 (CH_2, C-15), 26.2 (CH_2, C-23), 28.3 (CH_2, C-16), 29.2 (CH, C-25), 31.7 (CH_2, C-2), 31.9 (CH_2, C-7), 31.9 (CH, C-8), 34.0 (CH_2, C-22), 36.2 (CH, C-20), 36.5 (C, C-10), 37.3 (CH_2, C-1), 39.8 (CH_2, C-12), 42.2 (C, C-13), 42.3 (CH_2, C-4), 45.9 (CH, C-24), 51.3 (CH, C-9), 56.1 (CH, C-17), 56.8 (CH, C-14), 71.8 (CH, C-3), 121.7 (CH, C-6), 140.8 (C, C-5).EIMS *m/z* (rel. intensity, %): 414 (13), 105 (24), 95 (26), 85 (15), 79 (26), 71 (22), 57 (77), 55 (100).HREIMS: *m/z* 414.3855 $[M]^+$ (calcd. for $C_{29}H_{50}O$, 414.38616, Δ 0.0006 ppm).

S3: ^{1}H-NMR spectrum of *β*-sitosterol (**2**) in $CDCl_3$ (500 MHz)

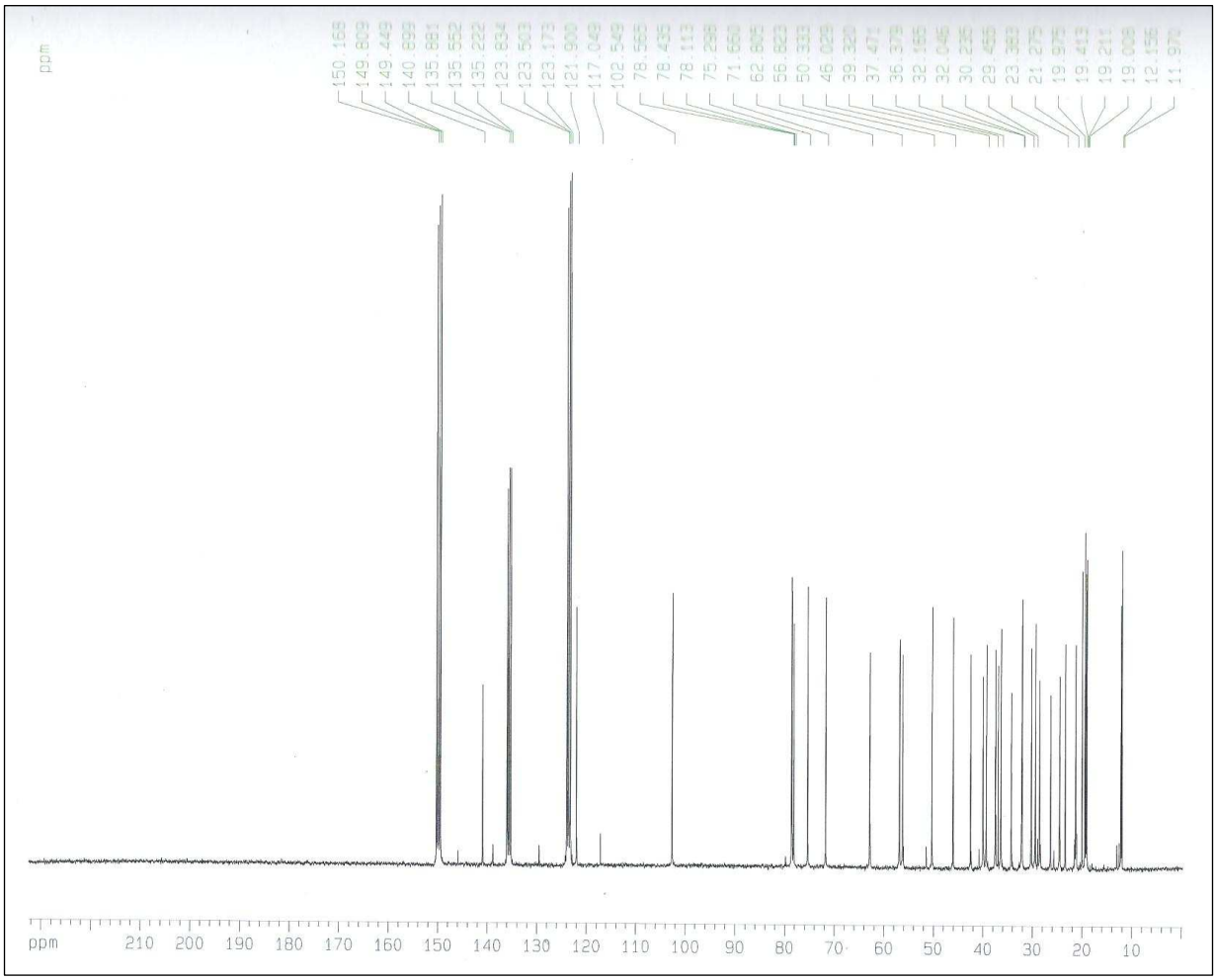


S4: ^{13}C NMR spectrum of *β*-sitosterol (**2**) in $CDCl_3$ (125 MHz)

*Stigmast-5-en-3-O-β-D-glucoside (**3**)*: White amorphous powder. ^{1}H NMR (C_5D_5N, 400 MHz) δ_H: 0.65 (3H, s, H-18), 0.84 (1H, m, H-9), 0.85 (6H, d, *J* = 7.2 Hz, H-26 & H-27), 0.89 (3H, t, *J* = 7.4 Hz, H-29), 0.92 (3H, s, H-19), 0.95 (1H, m, H-14), 0.98 (3H, d. *J* = 6.5 Hz, H-21), 0.99 (1H, m, H-1a), 1.01 (1H, m, H-24), 1.03 (1H, m, H-15a), 1.10 (1H, m, H-12a), 1.09 (1H, m, H-17), 1.10 (1H, m, H-22b), 1.26 (1H, m, H-16a), 1.26 (2H, m, H-23a,b), 1.30 (2H, m, H-28a,b),1.37 (1-H, m, H-8), 1.38 (1H, m, H-20), 1.41 (3H, m, H-11a,b, H-22a), 1.55 (2H, m, H-7a, H-15b), 1.69 (1H, m, H-25), 1.73 (1H, m, H-1b), 1.74 (1H, m, H-2a), 1.85 (1H, m, H-16b), 1.96 (2H, m, H-7b, H-12b), 2.10 (1H, m, H-2b), 2.46 (1H, t, *J* = 10.2 Hz, H-4b), 2.70 (1H, m, H-4a), 3.94 (1H, m, H-3, H-5′), 4.03 (1H, t, *J* = 8.0 Hz, H-2′), 4.27 (2H, t, , *J* = 8.0 Hz, H-3′,4′), 4.38 (1H, dd, *J* = 5.2 Hz, 11.8 Hz, , H-6′b), 4.53 (1H, dd, *J* = 2.2 Hz, 11.8 Hz, H-6′a), 5.02 (1H, d, *J* = 7.7, H-1′), 5.33 (1H, br.d, *J* = 2.2 Hz, H-6). ^{13}C NMR (C_5D_5N, 75 MHz) δ_C: 12.0 (CH_3, C-18), 12.2 (CH_3, C-29), 19.0 (CH_3, C-21), 19.2 (CH_3, C-26), 19.4 (CH_3, C-19), 20.0 (CH_3, C-27), 21.3 (CH_2, C-11), 23.4 (CH_2, C-28), 24.5 (CH_2, C-15), 26.4 (CH_2, C-23), 28.5 (CH_2, C-16), 29.5 (CH, C-25), 30.2 (CH_2, C-2), 32.0 (CH, C-8), 32.2 (CH_2, C-7), 34.2 (CH_2, C-22), 36.4 (C, C-10), 36.4 (CH, C-20), 37.5 (CH_2, C-1), 39.3 (CH_2, C-4), 39.9 (CH_2, C-12), 42.5 (C, C-13), 46.0 (CH, C-24), 50.3 (CH, C-9), 56.2 (CH, C-17), 56.8 (CH, C-14), 62.8 (CH_2, C-6′), 71.7 (CH, C-4′), 75.3 (CH, C-2′), 78.1 (CH, C-5′), 78.4 (CH, C-3), 78.6 (CH, C-3′), 102.5 (CH, C-1′), 121.9 (CH, C-6), 140.9 (C, C-5).EIMS *m/z* (rel. intensity, %): 396 (19), 255 (04), 135 (05), 87 (11), 85 (71), 83 (100), 69 (16), 55 (23).HREIMS: 415.3979 $[M^{-}C_6H_{11}O_5]^{+}$, $C_{29}H_{51}O$.

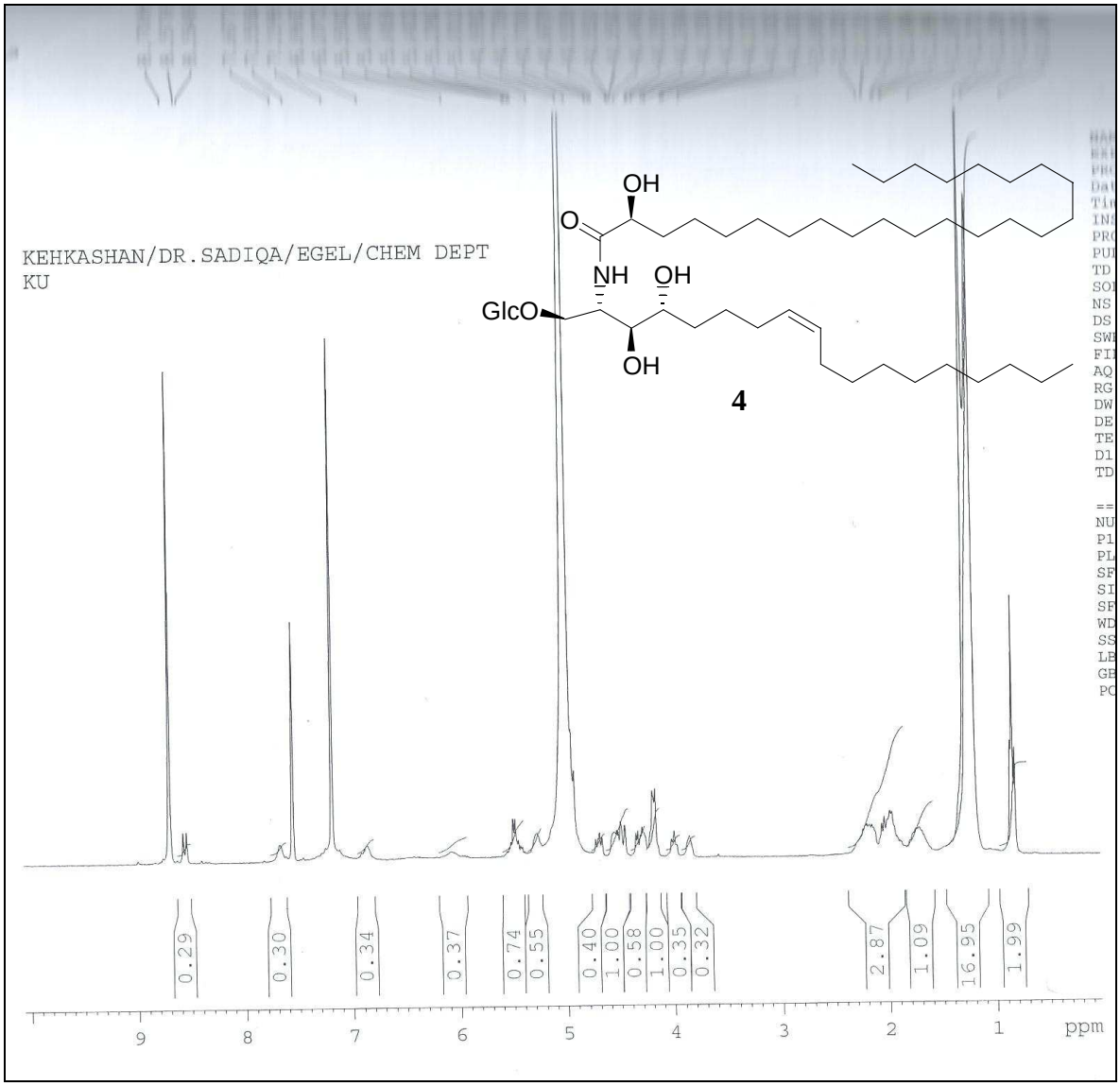


S5: ^{1}H-NMR spectrum of stigmast-5-en-3-*O*-*β*-D-glucoside (**3**) in C_5D_5N (400 MHz)

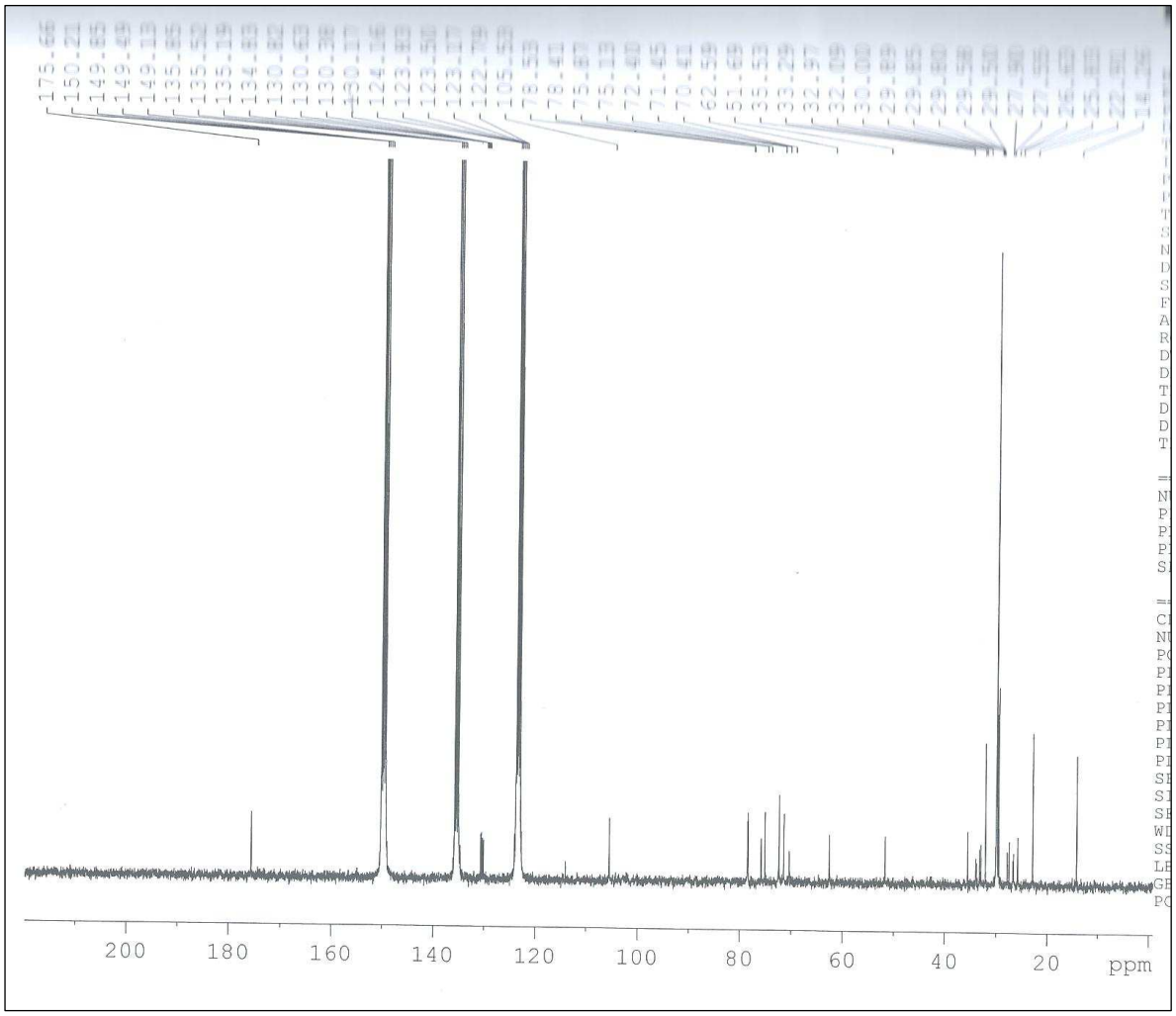


S6: ^{13}C-NMR spectrum of stigmast-5-en-3-*O*-*β*-D-glucoside (**3**) in C_5D_5N (75 MHz)

*(2S,1′S,2′S,3′R,7′Z)-N-1′-(O-β-D-glucopyranosyl)methyl-2′,3′-dihydroxy-heptadec-7′-enyl-2-hydroxytetracosaneamide (**4**)*:White gelatin.^{1}H NMR:(C_5D_5N, 300 MHz) δ_H: 0.85 (6H, t, *J* = 6.9 Hz, H-18, 24′), 1.29 (36H, br, d, H-4′-H-21′), 1.72 (6H, m, H-6, H-22′, H-23′), 1.98 (1H, m, H-5b), 2.05 (3H, m, H-3′, H-5a), 2.18 (4H, m, H-7,10), 3.86 (1H, m, H-5″), 4.01 (1H, t, *J* = 8.0 Hz, H-2″), 4.19 (3H, m, H-4, H-3″, H-4″), 4.30 (1H, m, H-3), 4.35 (1H, dd, *J* = 5.1 Hz, 12.0 Hz, H-6″a), 4.50 (1H, m, H-6″b), 4.52 (1H, m, H-1a), 4.57 (1H, m, H-2′), 4.70 (1H, dd, *J* = 6.6 Hz, 10.8 Hz, H-1b), 4.94 (1H, d, *J* = 8.0 Hz, H-1″) 5.28 (1H, m, H-2), 5.50 (2H, m, H-8, H-9), 8.56 (1H, d, *J* = 9.0 Hz, NH).^{13}C NMR (C_5D_5N, 75 MHz) δ_C: 14.3 (CH_3, C-18, 24′), 22.9 (CH_2, C-23′), 26.8 (CH_2, C-6), 27.6 (CH_2, C-10), 27.9 (CH_2, C-7), 29.5-30.0 (CH_2, C-4′-C-21′), 32.1 (CH_2, C-22′), 34.0 (CH_2, C-5), 35.5 (CH_2, C-3′), 51.7 (CH, C-2), 62.6 (CH_2, C-6″), 70.4 (CH_2, C-1), 71.5 (CH, C-4″), 72.4 (CH, C-4, C-2′), 75.1 (CH, C-2″), 75.9 (CH, C-3), 78.4 (CH, C-5″), 78.5 (CH, C-3″), 105.5 (CH, C-1″), 130.2 (CH, C-9), 130.4 (CH, C-8), 175.7 (C, C-1′).EIMS *m/z* (rel. intensity, %): 663 (84), 632 (17), 463 (14), 439 (21) 422 (16), 408 (53), 355 (43), 248 (61).ESI-MS m/z: 844.7004 $[M + H]^+$.

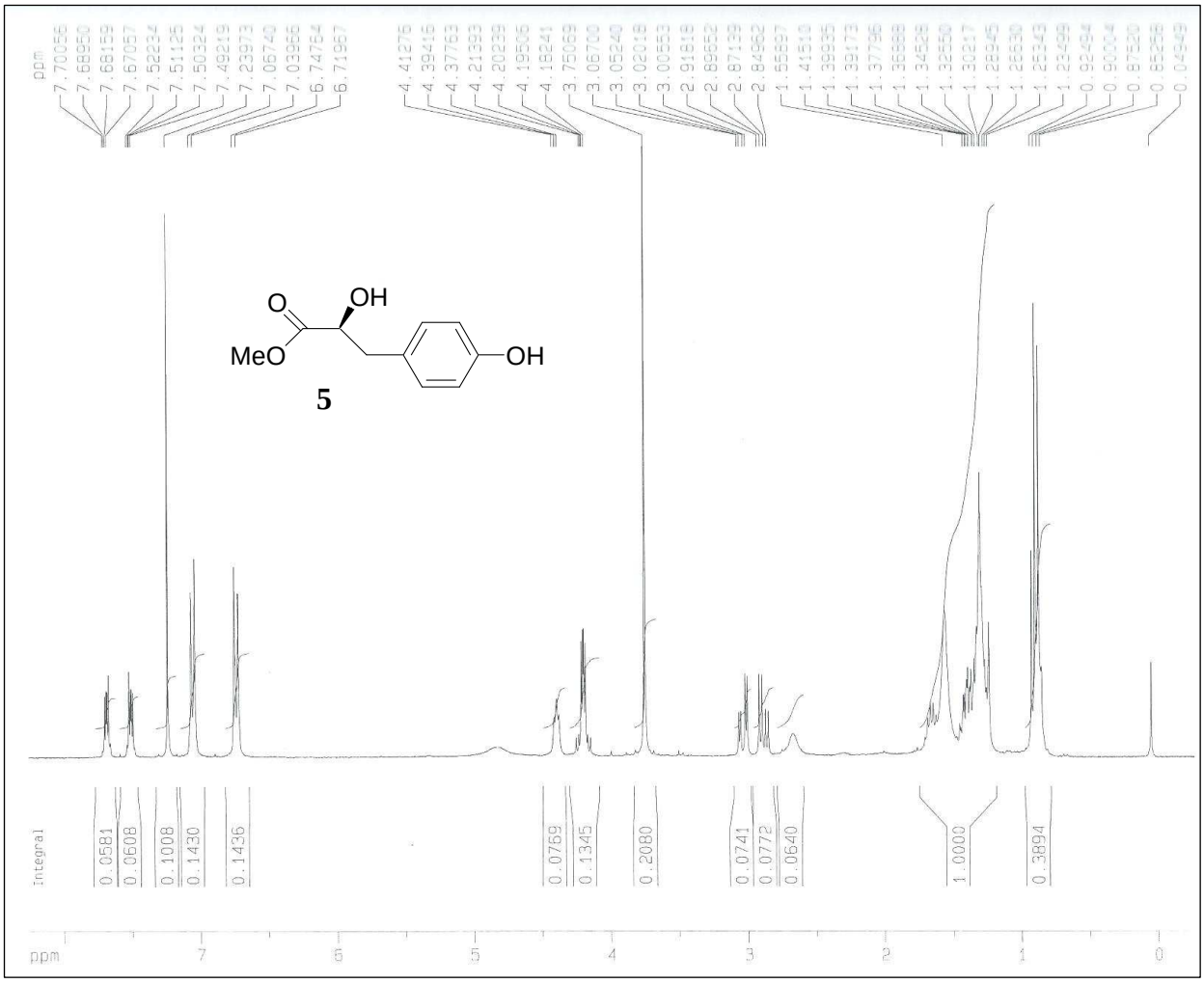


S7: ^{1}H-NMR spectrum of (2*S*,1′*S*,2′*S*,3′*R*,7′*Z*)-N-1′-(*O*-*β*-D-glucopyranosyl)methyl-2′,3′-dihydroxy-heptadec-7′-enyl-2-hydroxytetracosaneamide (**4**) in C_5D_5N (300 MHz)

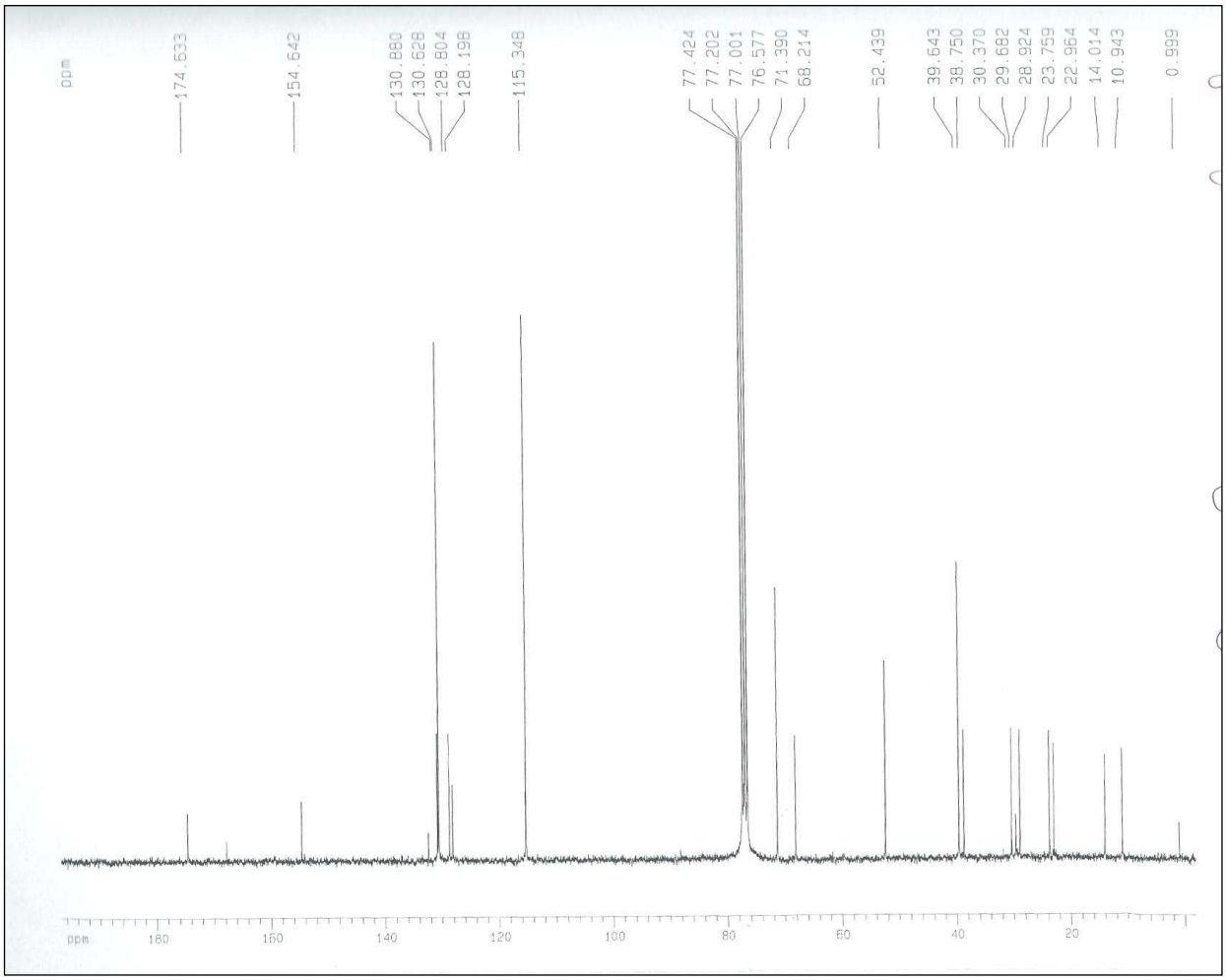


S8: ^{13}C-NMR spectrum of (2*S*,1′*S*,2′*S*,3′*R*,7′*Z*)-N-1′-(*O*-*β*-D-glucopyranosyl)methyl-2′,3′-dihydroxy-heptadec-7′-enyl-2-hydroxytetracosaneamide (**4**) in C_5D_5N (75 MHz)

*Methyl 2-hydroxy-3-(4′-hydroxy)-phenyl propionate (**5**)*:Amorphous powder. IR ν_{max} ($CDCl_3$) cm^{-1}: 3359, 1730, 1608, 1514.^{1}H NMR ($CDCl_3$, 300 MHz) δ_H: 2.88 (1H, dd, *J* = 6.5 Hz, 14.0 Hz, H-3a), 3.04 (1H, dd, *J* = 4.4 Hz, 14.0 Hz, H-3b), 3.75 (3H, s, 1-OCH_3), 4.39 (1H, br.t, *J* = 5.6 Hz, H-2), 6.73 (2H, d, *J* = 8.4 Hz, H-6, H-8), 7.05 (2H, d, *J* = 8.3 Hz, H-5, H-9).^{13}C NMR ($CDCl_3$,75 MHz) δ_C : 39.6 (CH_2, C-3), 52.4 (CH_3, 1-OCH_3), 71.4 (CH, C-2), 115.3 (CH, C-6/C-8), 128.2 (C, C-4), 130.6 (CH, C-5/C-9), 154.6 (C, C-7), 174.6 (C, C-1).EIMS *m/z* (rel. intensity, %): 196 (02), 178 (02), 108 (09), 107 (100), 91 (08), 83 (05), 77 (11), 65 (02).HREIMS: 196.0747 $[M]^+$ (calcd. for $C_{10}H_{12}O_4$, 196.07354, Δ 0.001 ppm).FAB MS (+) *m/z*: 197 $[M + H]^+$, $C_{10}H_{13}O_4$,CI (+): 197 [M + H].

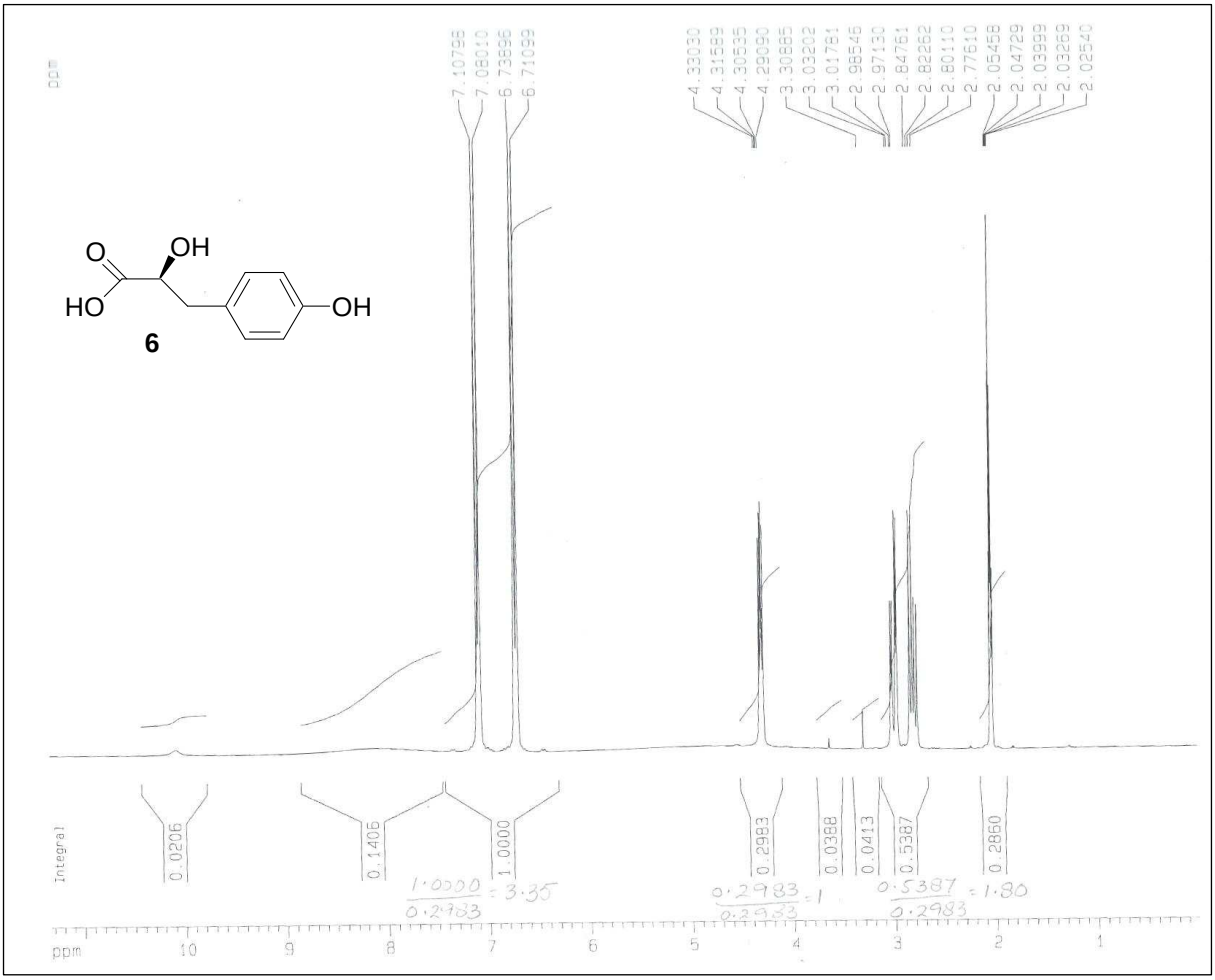


S9: ^{1}H-NMR spectrum of methyl 2-hydroxy-3-(4′-hydroxy)-phenyl propionate (**5**) in $CDCl_3$ (300 MHz)

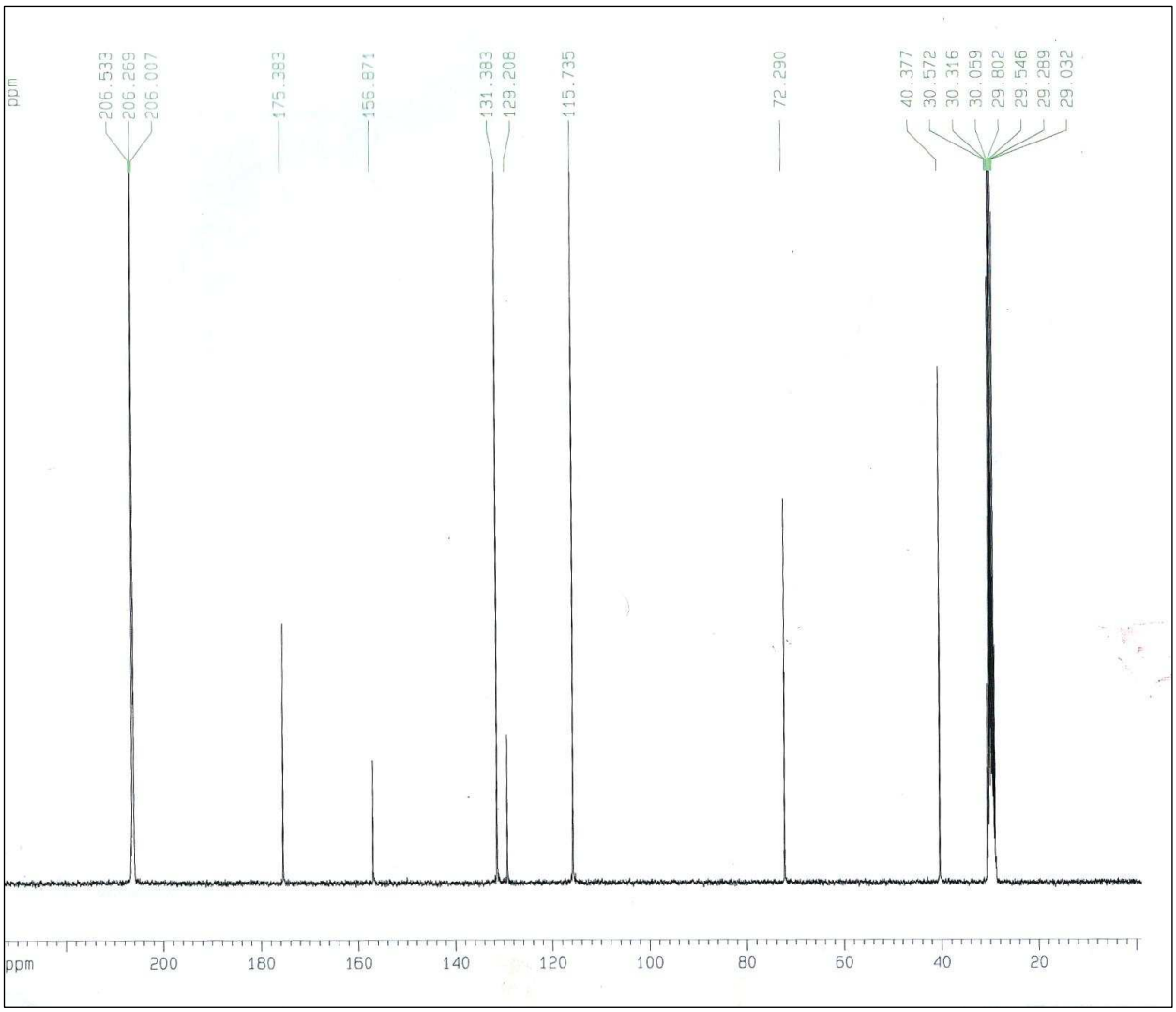


S10: ^{13}C-NMR spectrum of methyl 2-hydroxy-3-(4′-hydroxy)-phenyl propionate (**5**) in $CDCl_3$ (75 MHz)

*(2R)-(4-hydroxyphenyl) lactic acid (**6**):*IR ν_{max} (KBr) cm^{-1}: 3466, 3235, 1738, 1613.^{1}H NMR (C_3D_6O, 300 MHz) δ_H: 2.81 (1H, dd, *J* = 7.5 Hz, 14.0 Hz, H-3a), 3.00 (1H, dd, *J* = 4.3 Hz, 14.0 Hz, H-3b), 4.31 (1H, dd, *J* = 4.3 Hz, 7.5 Hz, H-2), 6.72 (2H, d, *J* = 8.4 Hz, H-6/H-8), 7.09 (2H, d, *J* = 8.4 Hz, H-5/H-9). ^{13}C NMR (C_3D_6O, 75 MHz) δ_C: 40.4 (CH_2, C-3), 72.3 (CH, C-2), 115.7 (CH, C-6/C-8), 129.2 (C, C-4), 131.4 (CH, C-5/C-9), 156.9 (C, C-7), 175.4 (C, C-1).EIMS *m/z* (rel. intensity, %): 182 (02), 107 (100), 91 (15), 79 (11), 78 (16), 77 (48), 65 (14), 53 (14). HREIMS: 182.0578 $[M]^+$ (calcd. for $C_9H_{10}O_4$, 182.05789, Δ 0.00009 ppm). CI (+): 183 $[M + H]^+$.

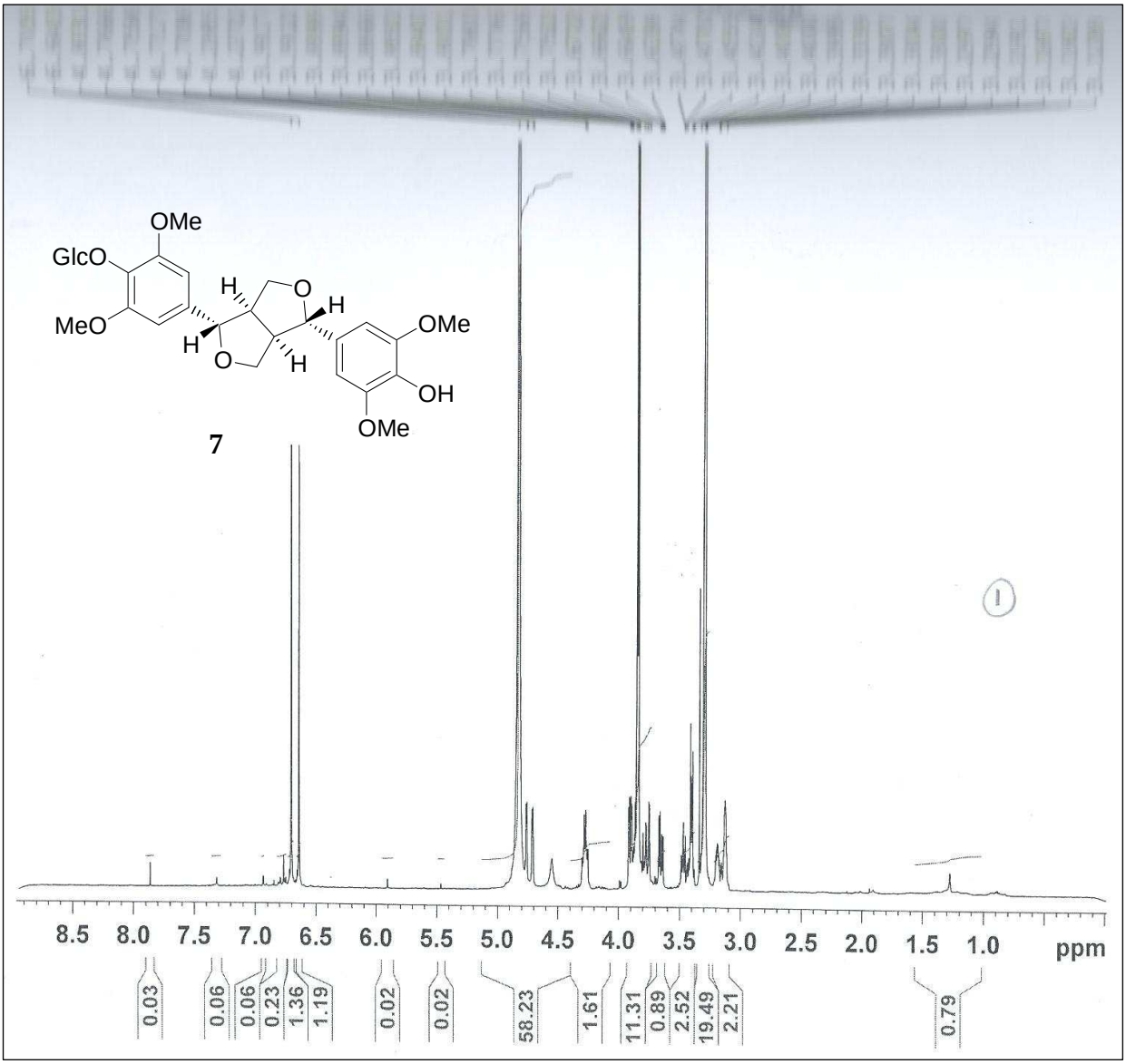


S11: ^{1}H-NMR spectrum of (2*R*)-(4-hydroxyphenyl) lactic acid (**6**) in C_3D_6O (300 MHz).

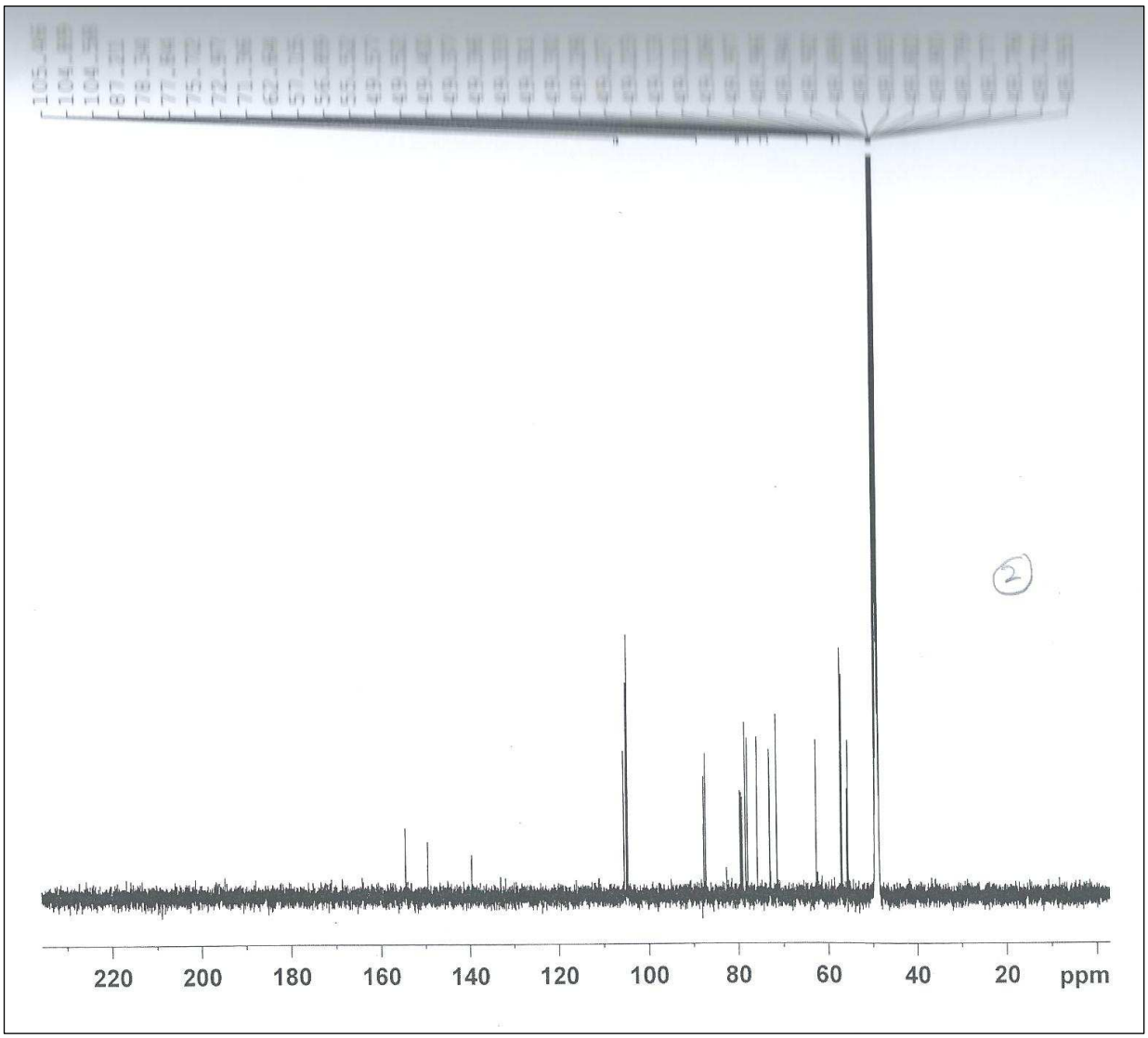


S12: ^{13}C-NMR spectrum of (2*R*)-(4-hydroxyphenyl) lactic acid (**6**) in C_3D_6O (75 MHz)

Syringaresinol mono-β-D-glucoside (7):Amorphous solid. ^{1}H NMR (CD_3OD, 500 MHz) δ_H: 3.13 (2H, m, H-1, H-5), 3.19 (1H, m, H-5″′), 3.40 (2H, m, H-3″′, H-4″′), 3.66 (1H, dd, *J* = 5.0 Hz, 11.5 Hz, H-6″′a), 3.77(1H, dd, *J* = 2.0 Hz, 12.0 Hz, H-6″′b), 3.84 (6H, br.s, 3″, 5″-OCH_3), 3.85 (6H, br.s, 3′, 5′- OCH_3), 3.91 (2H, dd, *J* = 2.5 Hz, 9.0Hz, H-4a, H-8a), 4.27 (2H,m, H-4b, H-8b), 4.71 (1H, d, *J* = 4.5Hz, H-6), 4.76 (1H, d, *J* = 4.0Hz, H-2), 4.83 (1H, br.s, H-1″′), 6.64 (2H, s, H-2″/H-6″), 6.71 (2H, s, H-2′/H-6′).^{13}C NMR (CD_3OD, 125 MHz) δ_C: 55.5 (CH, C-5), 55.7 (CH, C-1), 56.9 (CH_3, C-3″, C-5″-OCH_3), 57.2 (CH_3, C-3′, C-5′-OCH_3), 62.6 (CH_2, C-6″′), 71.4 (CH, C-4″′), 72.9 (CH_2, C-4), 73.0 (CH_2, C-8), 75.7 (CH, C-2″′), 77.8 (CH, C-3″′), 78.3 (CH, C-5″′), 87.2 (CH, C-2), 87.6 (CH, C-6), 104.6 (CH, C-2″/C-6″), 104.9 (CH, C-2′/C-6′), 105.5 (CH, C-1″′), 132.0 (C, C-1″), 133.0 (C, C-4″) 133.1 (C, C-4′), 149.4 (C, C-3″/C-5″), 154.4 (C, C-3′/C-5′).ESIMS: 579.1988.

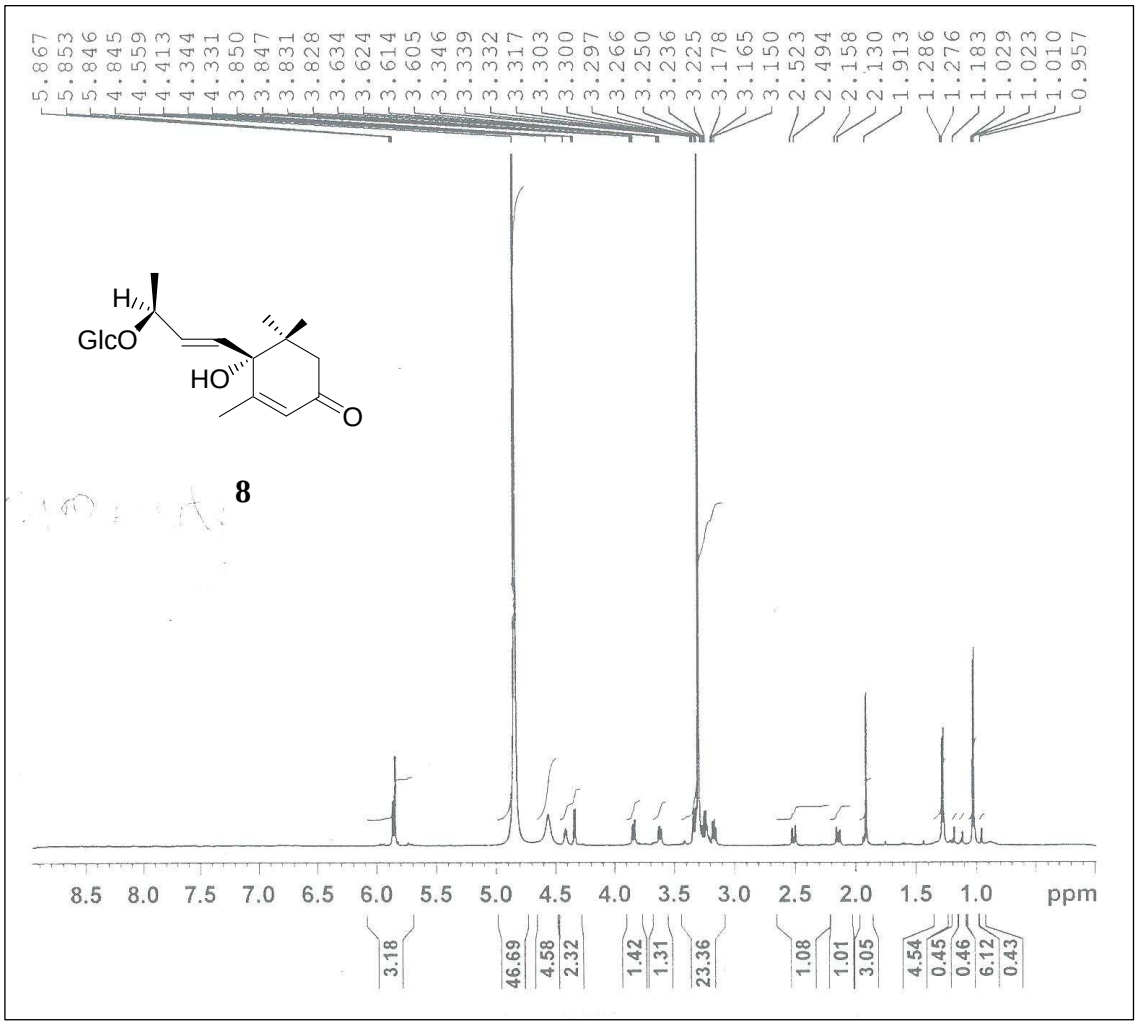


S13: ^{1}H-NMR spectrum of syringaresinol mono-*β*-D-glucoside (**7**) in CD_3OD (500 MHz)

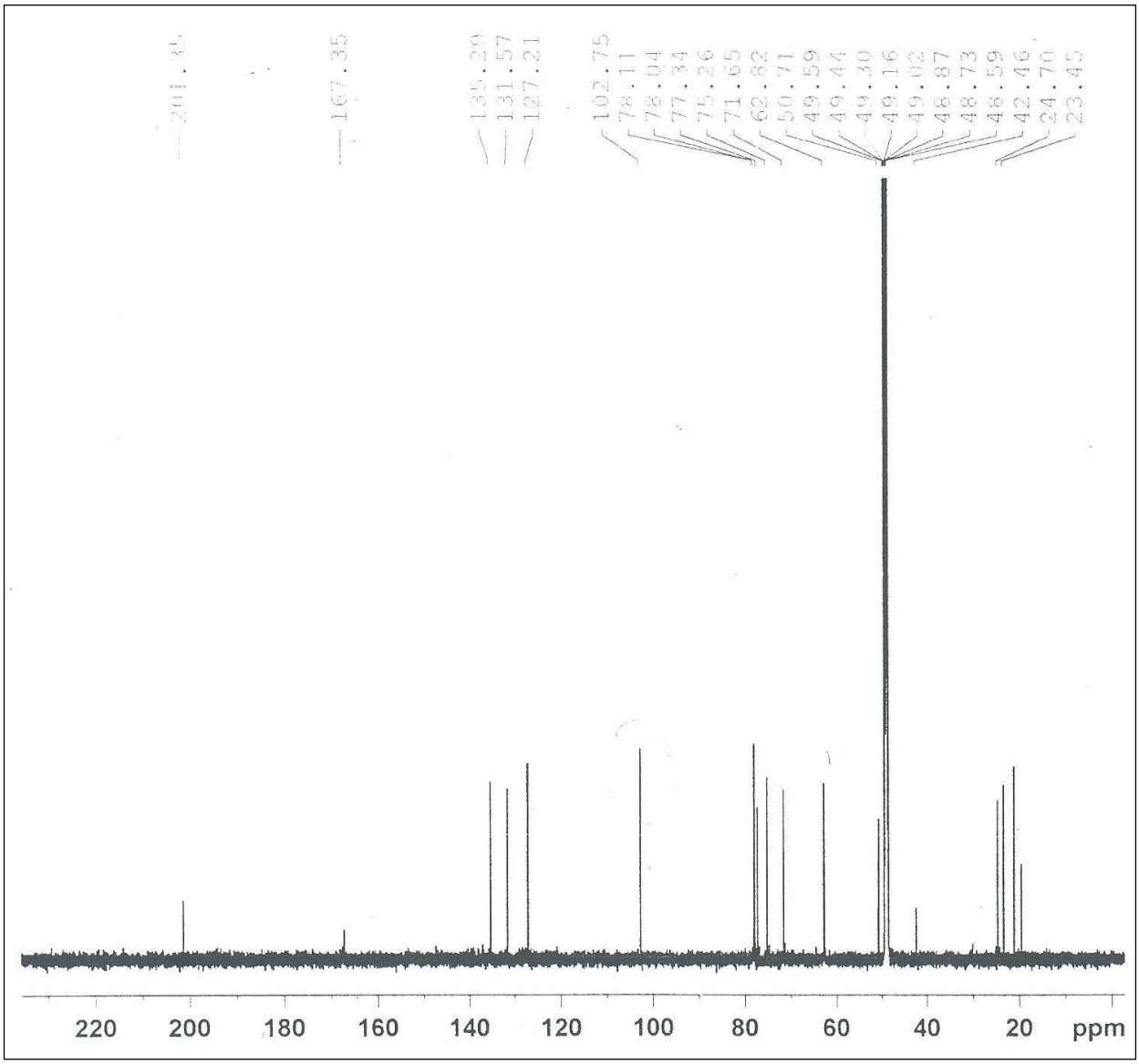


S14: ^{13}C-NMR spectrum of syringaresinol mono-*β*-D-glucoside (**7**) in CD_3OD (125 MHz)

*6-Hydroxy-3-oxo-α-ionol 9-O-β-D-glucopyranoside (**8**)*:[1]H NMR (CD_3OD, 600 MHz) δ_H: 1.02 (3H, s, H-12), 1.03 (3H, s, H-11), 1.29 (3H, d, *J* = 6.0 Hz, H-10), 1.94 (3H, s, H-13), 2.14 (1H, d, *J* = 16.8 Hz, H-2a), 2.51 (1H, d, *J* = 16.8 Hz, H-2b), 3.17 (1H, dd, *J* = 7.5 Hz, 9.0 Hz, H-2′), 3.22 (1H, m, H-5′), 3.25 (1H, m, H-4′), 3.34 (1H, m, H-3′), 3.63 (1H, dd, *J* = 6.0 Hz, 12.0 Hz, H-6′a), 3.85 (1H, dd, *J* = 1.8 Hz, 12.0 Hz, H- 6′b), 4.34 (1H, d, *J* = 7.8 Hz, H-1′), 4.56 (1H, br. s, H-9), 5.84 (1H, s, H-4), 5.85 (1H, d, *J* = 12.6 Hz, H-7), 5.86 (1H, dd, *J* = 8.4 Hz, 12.6 Hz, H-8). [13]C NMR (CD_3OD, 150 MHz) δ_C: 19.6 (CH_3, C-13), 21.2 (CH_3, C-10), 23.5 (CH_3, C-11), 24.7 (CH_3, C-12), 42.5 (C, C-1), 50.7 (CH_2, C-2), 62.8 (CH_2, C-6′), 71.7 (CH, C-4′), 75.3 (CH, C-2′), 77.3 (CH, C-9), 78.0 (CH, C-5′), 78.1 (CH, C-3′), 80.0 (C, C-6), 102.8 (CH, C-1′), 127.2 (CH, C-4), 131.6 (CH, C-7), 135.3 (CH, C-8), 167.4 (C, C-5), 201.4 (C, C-3).EIMS *m/z* (rel. intensity, %): 224 (08), 207 (33), 166 (16), 149 (100), 135 (25), 124 (79), 123 (43), 106 (25).FAB MS (+) *m/z*: 387 $[M + H]^+$, $C_{19}H_{31}O_8$ESI-MS m/z: 387.2063 $[M + H]^+$ (calcd. for $C_{19}H_{31}O_8$, 387.20186, Δ 0.0044 ppm).

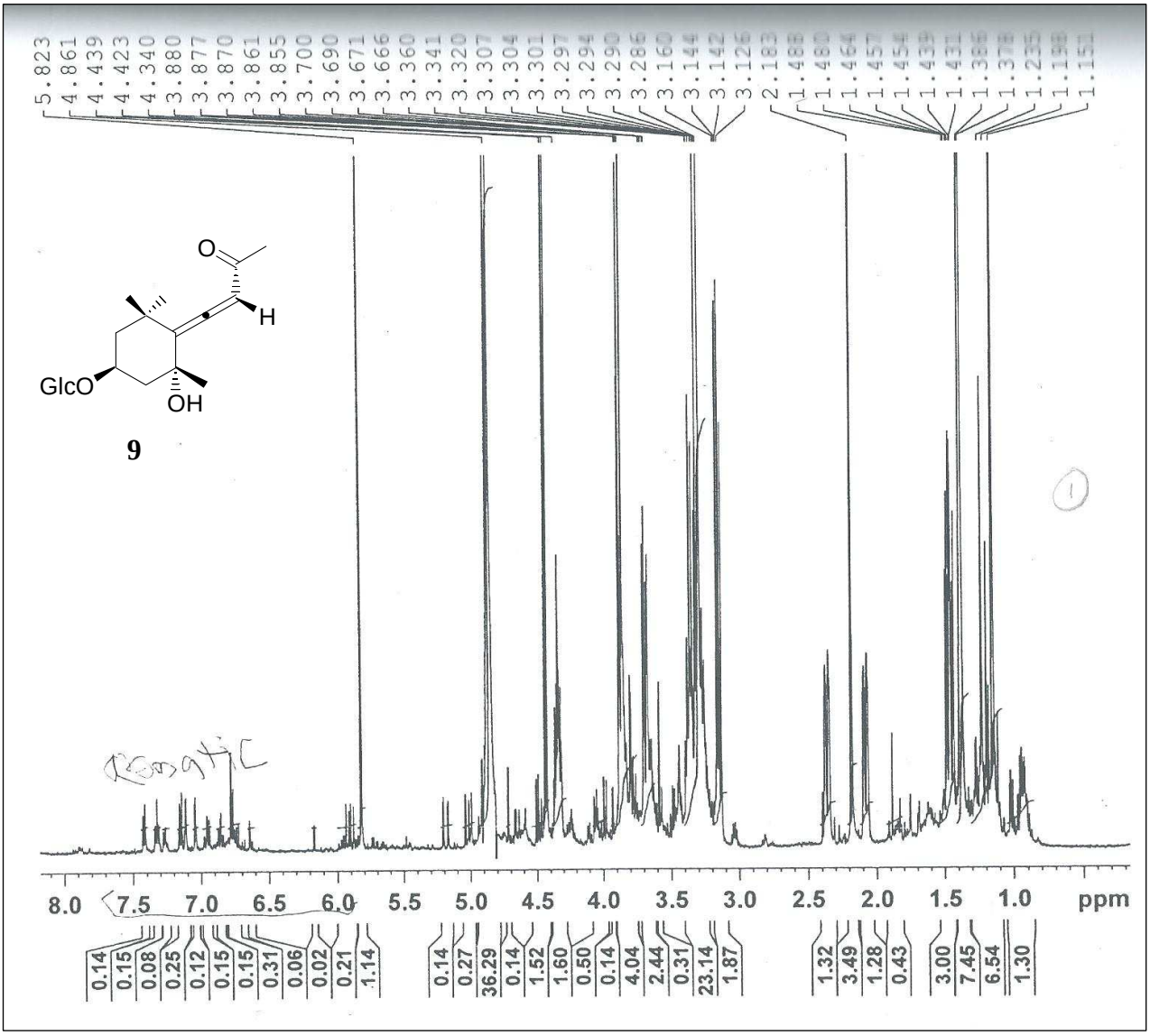


S15: [1]H-NMR spectrum of 6-hydroxy-3-oxo-*α*-ionol 9-*O*-*β*-D-glucopyranoside (**8**) in CD_3OD (600 MHz)



S16: ^{13}C-NMR spectrum of 6-hydroxy-3-oxo-*α*-ionol 9-*O*-*β*-D-glucopyranoside (**8**) in CD_3OD (150 MHz)

*Staphylionoside D (**9**)*:Amorphous powder. ^{1}H NMR (CD_3OD, 500 MHz) δ_H: 1.15 (3H, s, H-12), 1.37 (3H, s, H-11), 1.38 (3H, s, H-13), 1.45 (2H, ddd, *J* = 4.0 Hz, 11.5 Hz, 13.0 Hz, H-2a, H-4a), 2.08 (1H, ddd, *J* = 2.0 Hz, 4.0 Hz, 13.0 Hz, H-4e), 2.18 (3H, s, H-10), 2.36 (1H, ddd, *J* = 2.0 Hz, 3.5 Hz, 13.0 Hz, H-2e), 3.14 (1H, dd, *J* = 8.0 Hz, 9.0 Hz, H-2′), 3.28 (1H, m, H-4′), 3.29 (1H, m, H-5′), 3.35 (1H, m, H-3′), 3.70 (1H, m, H-6′a), 3.88 (1H, m, H-6′b), 4.35 (1H, tt, *J* = 4.0 Hz, 11.5 Hz, H-3), 4.46 (1H, d, *J* = 8.0 Hz, H-1′), 5.83 (1H, s, H-8). ^{13}C NMR (CD_3OD, 125 MHz) δ_C: 26.6 (CH_3, C-10), 29.4 (CH_3, C-11), 30.8 (CH_3, C-13), 32.3 (CH_3, C-12), 37.0 (C, C-1), 46.6 (CH_2, C-2), 48.1 (CH_2, C-4), 62.7 (CH_2, C-6′), 71.6 (CH, C-4′), 72.4 (C, C-5), 72.6 (CH, C-3), 75.1 (CH, C-2′), 77.9 (CH, C-5′), 78.1 (CH, C-3′), 101.2 (CH, C-8), 102.7 (CH, C-1′), 120.1 (C, C-6), 200.9 (C, C-7), 211.5 (C, C-9).EIMS *m/z* (rel. intensity, %): 118 (03), 87 (12), 85 (83), 84 (04), 83 (100), 82 (05), 78 (17), 63 (27).FAB MS (+) *m/z*: 387 $[M + H]^+$, $C_{19}H_{31}O_8$ ESI-MS m/z: 387.1952 $[M + H]^+$ (calcd. for $C_{19}H_{31}O_8$, 387.20186, Δ 0.0066 ppm).



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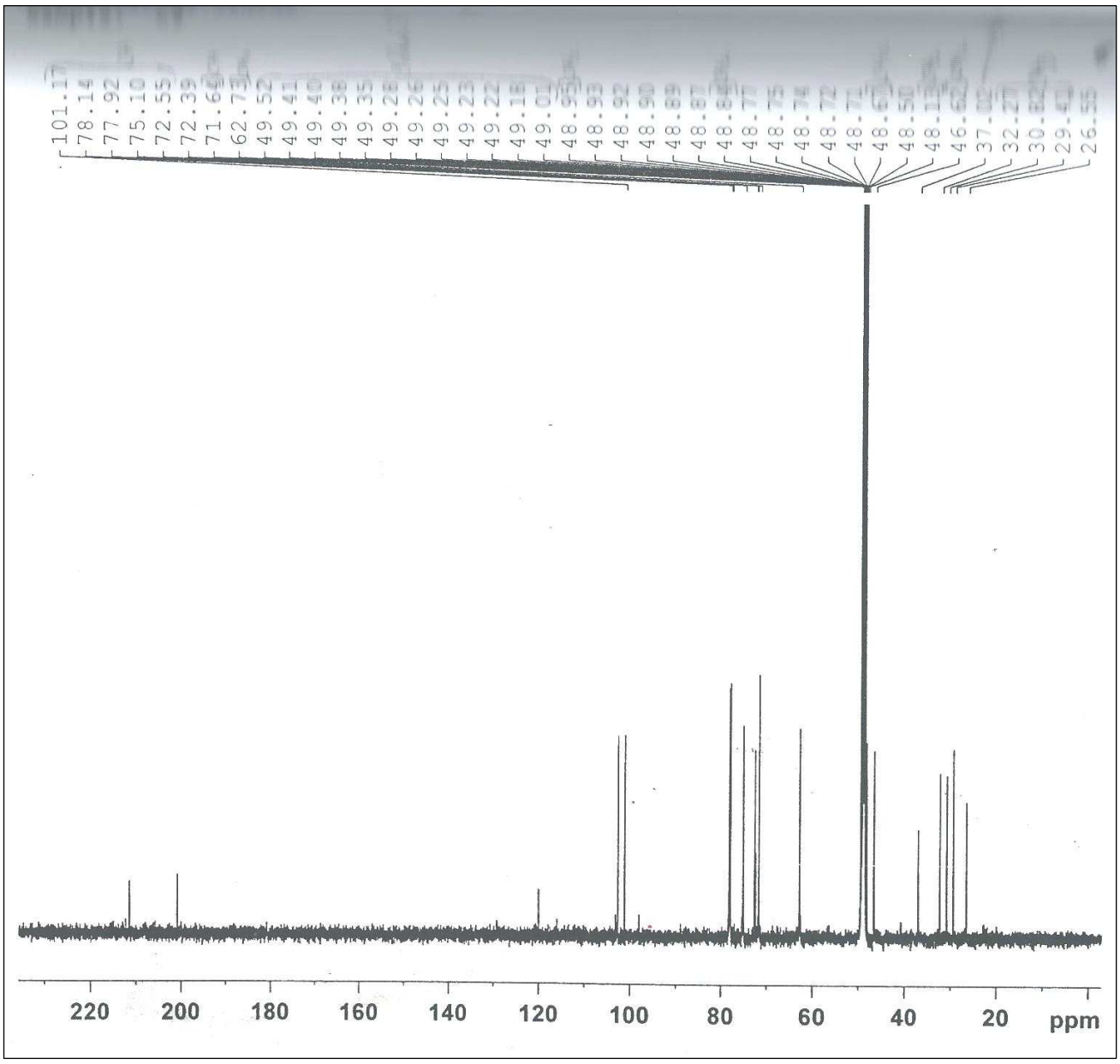
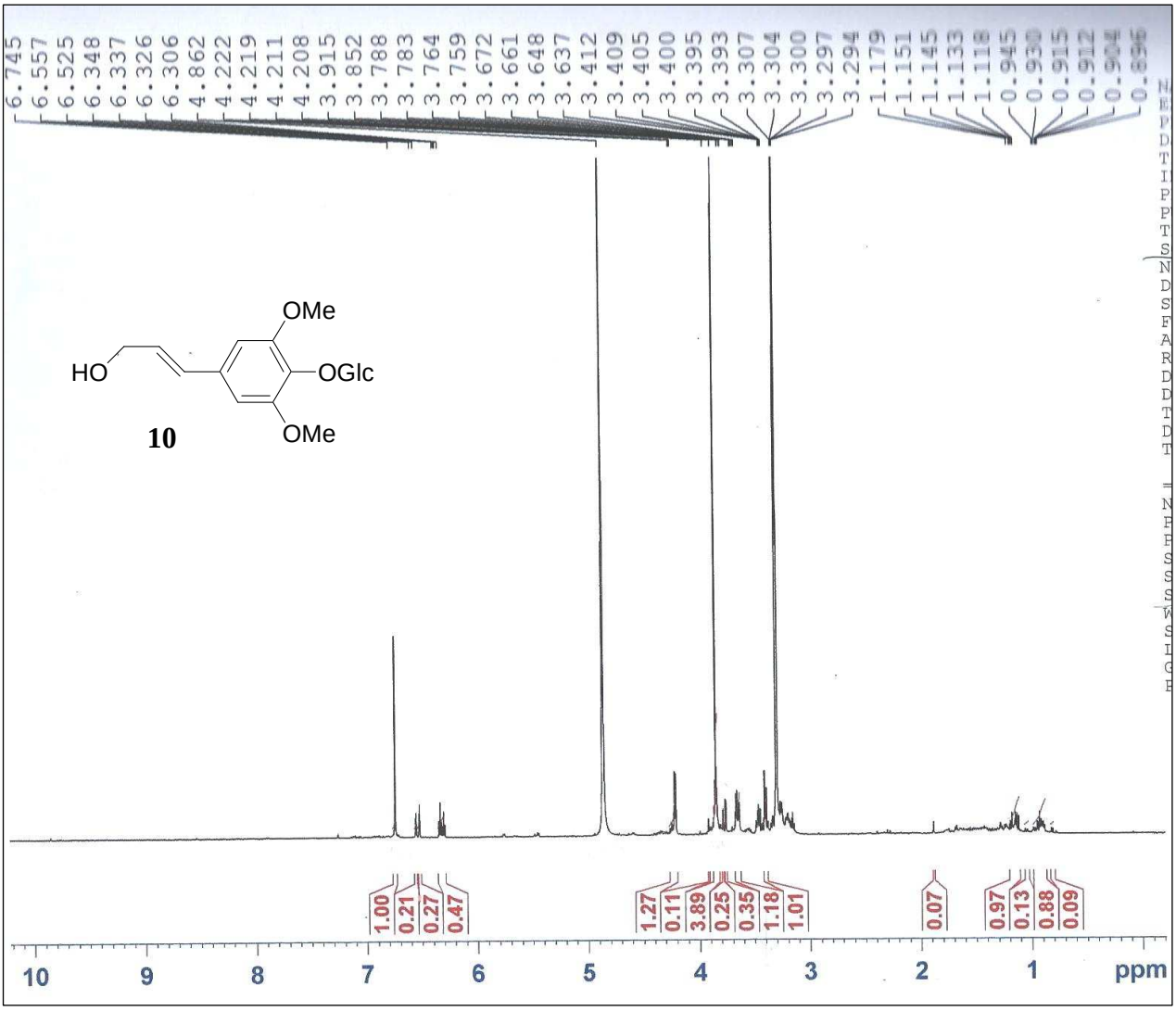
S17: ^{1}H-NMR spectrum of staphylionoside D (**9**) in CD_3OD (500 MHz)

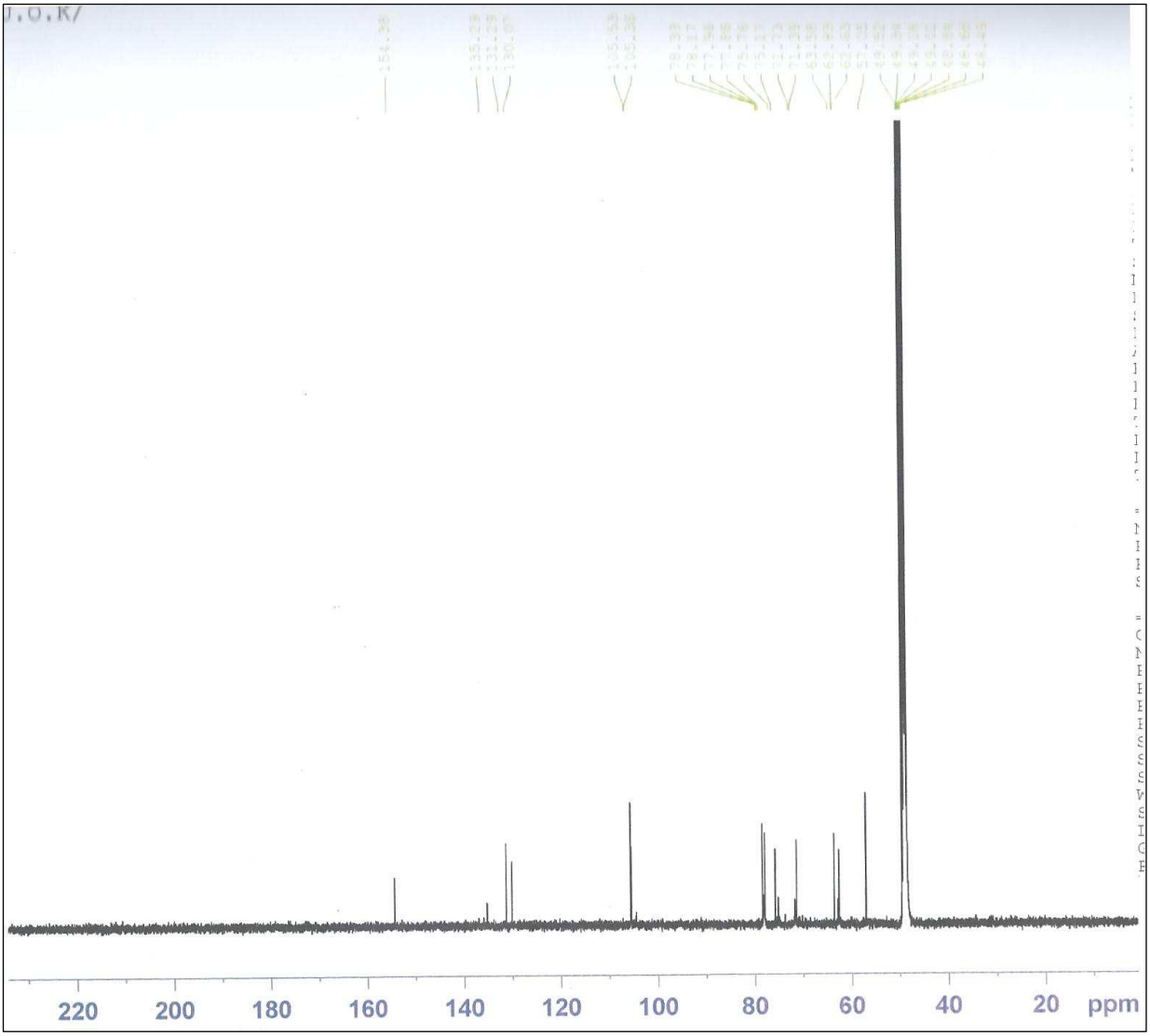


S18: ^{13}C-NMR spectrum of staphylionoside D (**9**) in CD_3OD (125 MHz)

*3-(3′, 5′-Dimethoxy-4′-O-β-*D*-glucopyranosyl-phenyl)-prop-2E-en-1-ol(**10**)*:[1]H NMR (CD_3OD, 500 MHz,) δ_H: 3.20 (1H, m, H-5′), 3.41 (2H, m, H-3′, H-4′), 3.47 (1H, m, H-2′), 3.65 (1H, dd, *J* = 5.5 Hz, 12.0 Hz, H-6′a), 3.77 (1H, dd, *J* = 2.5 Hz, 12.0 Hz, H-6′b), 3.85 (3H, s, 3/5-OCH_3), 4.22 (2H, dd, *J* = 1.5 Hz, 5.5 Hz, H-9), 4.86 (1H, d, *J* = 7.8 Hz, H-1′), 6.32 (1H, dt, *J* = 5.5 Hz, 16.0 Hz, H-8), 6.54 (1H, br.d, *J* = 16.0 Hz, H-7), 6.75 (2H, s, H-2/H-6). [13]C NMR (CD_3OD, 125 MHz) δ_C: 57.1 (CH_3, 3, 5-OCH_3), 62.6 (CH_2, C-6′), 63.6 (CH_2, C-9), 71.4 (CH, C-4′), 75.7 (CH, C-2′), 77.9 (CH, C-5′), 78.4 (CH, C-3′), 105.3 (CH, C-1′), 105.5 (CH, C-2/C-6), 130.1 (CH, C-8), 131.3 (CH, C-7), 135.3 (C, C-4), 154.4 (CH_3, C-3/C-5).EIMS *m/z* (rel. intensity, %): 145 (12), 127 (11), 115 (21), 101 (22), 99 (20), 87 (19), 85 (29), 73 (100). FAB MS (+) *m/z*: 373 $[M + H]^+$, $C_{17}H_{25}O_9$.

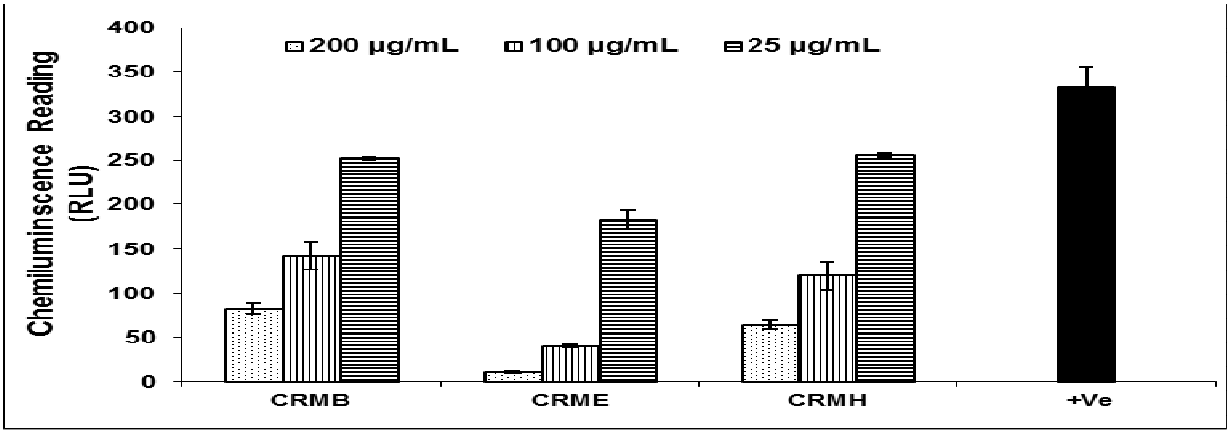


S19: [1]H-NMR spectrum of 3-(3′, 5′-dimethoxy-4′-*O-β-*D-glucopyranosyl-phenyl)-prop-2*E*-en-1-ol (**10**) in CD_3OD (500 MHz)



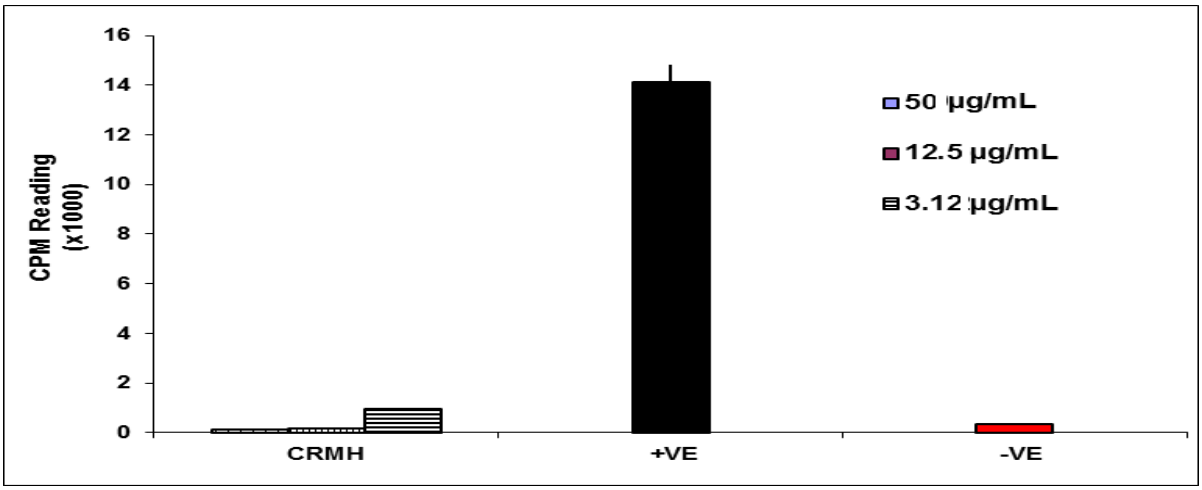
S20: ^{13}C-NMR spectrum of 3-(3′, 5′-dimethoxy-4′-*O*-*β*-D-glucopyranosyl-phenyl)-prop-2*E*-en-1-ol (**10**) in CD_3OD (125 MHz)

S21: (Figure 2) Effect of fractions on ROS production by whole blood phagocytes determined by chemiluminescence technique and oxidative burst study.



RLU = relative light unit

S22: (Figure 3) Effect of CRMH on phytohamagglutinin (PHA-P) dependant T-cell proliferation.



CPM = counts per minute

+ve = Cell activated with PHA, -ve = Cell without PHA activation

Isolation of human polymorphoneutrophils (PMNs)

Heparinized blood was obtained by vein puncture aseptically from healthy volunteers (25 – 38 years age). The buffy coat containing PMNs were collected by dextran sedimentation and cells were isolated after the lymphocyte separation medium (LSM) (MP Biomedicals, Ohio, USA) density gradient centrifugation. PMNs were collected from bottom of the tube. Cells were washed twice and suspended in Hank's Balance Salt Solution [Ca and Mg free] ($HBSS^{--}$) pH, 7.4 (Sigma-Aldrich Steinheim, Germany). Neutrophils were purified from RBCs using hypotonic solution. Cells were adjusted to their required concentration using Hank's Balance Salt Solution Ca^{+2} and Mg^{+2} ($HBSS^{++}$) obtained from Sigma-Aldrich, Steinheim, Germany [23].

Chemiluminescence assay

Luminol-enhanced chemiluminescence assay was performed following Waleed et al.[24]. 25 µL of diluted (1:50) whole blood in $HBSS^{++}$ was incubated with 25 µL of serially diluted compounds. The concentration ranges between 1 to 100 µg/mL. Control wells contain $HBSS^{++}$and the diluted whole blood but no compound. Test was performed in Corning White 96 wells plates (New York, USA). Culture was incubated at 37 °C for 20 min in the thermostated chamber of the lab system luminoscan RS (Vienna, Virginia). 25 µL of opsonized zymosan-A (*Saccharomyces cerevisiae* origin), followed by 25 µL luminol (7×10^{-5} M) (Alfa Aesar, Karlsruhe, Germany) along with $HBSS^{++}$ were added to each well to obtain a 100 µL volume/well. The luminometer results were recorded in Relative Light Unit (RLU).

Nitrite concentration in mouse macrophage culture medium

The mouse macrophage cell line J774.2 (European Collection of Cell Cultures, UK) was cultured in IWAKI's 75 cc flask (Asahi Techno Glass, Tokyo, Japan) in Dulbecco's Modified Eagle Medium (DMEM) (Sigma-Aldrich, Steinheim, Germany) that contain 10% fetal bovine serum (GIBCO, New York, USA) supplemented with 1% streptomycin/penicillin. Flasks were kept at 37 °C in humidified air containing 5% CO_2. Cells (10^6 cells/mL) were then transferred to a 24-well plate. The Nitric oxide synthase (NOS-2) in macrophages was induced by the addition of 30 µg/mL *E. coli* lipopolysaccharide (LPS) (DIFCO Laboratories Michigan, USA). The test compounds were added at 25 µg/mL concentration. Soon after LPS stimulation cells were re-incubated at 37 °C in 5% CO_2. Finally the cell culture supernatant was collected after 48 h for analysis. Nitrite accumulation in cell culture supernatant was measured [25]. In brief 50 µL of 1% sulfhanilamide in 2.5% phosphoric acid and 50 µL of 0.1% naphtyl-ethylenediamine dihydrochloride in 2.5% phosphoric acid were added to 50 µL of culture medium. After 10 min of incubation at room temperature the absorbance was read at 550 nm. Micro molar concentrations of nitrite were calculated from a standard curve, which was generated using sodium nitrite as reference compound.

T-cell proliferation assay

Cell proliferation was evaluated by standard thymidine incorporation assay following a reported method [26]. Briefly, cells were obtained from peripheral blood of healthy individuals and then cultured at a concentration of 5×10^5/mL in a 96-well round bottom IWAKI's tissue culture plates (Asahi Techno Glass, Tokyo, Japan). Preliminary experiments were conducted to determine the optimum concentration of PHA on T-cell proliferation. PHA concentration of 5µg/mL was found to be optimum and hence used in experiments. Cells were stimulated with 5 g/mL of PHA-P (Sigma Co. St. Louis, USA). 200 µg/mL concentrations of compounds were added, each in triplicate. The plates were incubated for 72 h at 37 °C in 5% CO_2 incubator. After 72 h, cultures were pulsed (0.5 µCi/well) with tritiated thymidine (Amersham Pharmacia Biotech, Buckinghamshire, UK) and further incubated for 18 h. Cells were harvested onto a glass fiber filter (Connectorate Dietikon, Switzerland) using cell harvester (Ionotech Dottikon, Switzerland). The tritiated thymidine incorporated into the cells was measured by a liquid scintillation counter (LS 6500, Beckman Coulter, USA). Results were expressed as mean count per minute (CPM). The inhibitory activity of compounds on T lymphocyte proliferation was calculated using the formula:

Inhibitory Activity (%) = [{Control group (CPM) – Experimental group (CPM)}/ Control group (CPM)] x 100

Statistical analyses

Results are expressed as mean % inhibition of three determinations at level of significance $P \leq 0.05$* $P \leq 0.005$** and is calculated using ANOVA.

References for supporting information

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