

SUPPLEMENTARY MATERIAL

Enzyme mediated-transesterification of verbascoside and evaluation of antifungal activity of synthesized analogues

Abstract: Enzymatic acylation of verbascoside, a polyhydroxylated natural product was reported in this study using five different commercial lipases and taking p-nitrophenyl alkanoates as acyl donors. Out of these enzymes, the immobilized *Candida antartica* lipase B was found as the enzyme of choice. Mono- and di-acylated products were formed, with mono as major product indicating high regio-selective nature of such transformations. A series of acyl esters of verbascoside have been synthesized by this enzymatic transesterification methodology. The lipophilicity of the synthesized analogues was also checked. The analogues were further subjected to synergistic antifungal activity with Amphotericin B (AmB) against *Candida albicans*. Four-fold reduction in minimum inhibitory concentration (MIC) of AmB was observed with few synthesized analogues such as verbascoside 4''-octanoate (**3b**), verbascoside 4''-palmitate (**3d**) and verbascoside 4'', 4'-dipalmitate (**4d**) at a concentration of 0.5 µg/ml.

Abbreviations:

DMAP:	Dimethyl amino pyridine
DCC:	Dicyclohexylcarbodiimide
DCM:	Dichloromethane
MIC:	Minimum Inhibitory concentration

Table S1

Table S1: C logP values of verbascoside and its acylated analogues

Compound	ClogP
verbascoside	-0.69
per acetylated verbascoside (2a)	6.24
per butyrate verbascoside (2b)	8.32
verbascoside 4'-butyrate (3a)	0.24
verbascoside 4''-octanoate (4a)	0.97
verbascoside 4''-4'-butyrate(3b)	1.41
verbascoside 4'',4'-dioctanoate (3b)	1.81
verbascoside 4''-myristylate (3c)	2.13
verbascoside 4''-palmitate (4c)	2.89
verbascoside 4'',4'-dimyristylate (3d)	2.42
verbascoside 4'',4'-dipalmitate (4d)	3.02

Compound Characterization S1

Per acetylated verbascoside (2a)

(Yield 41%); Colourless solid; mp: 158 °C; ¹H NMR (CDCl₃, 500 MHz): δ 7.71 (d, 1H, *J* = 16 Hz), 7.35 (d, 2H, *J* = 8.5 Hz), 7.25 (d, 2H, *J* = 7.8 Hz), 7.00 (d, 3H, *J* = 10 Hz), 6.43 (d, 1H, *J* = 15.98 Hz), 5.15 (m, 6H), 4.45 (d, 1H, *J* = 8.01 Hz), 4.14 (s, 3H), 3.92 (m, 2H), 3.73 (d, 2H, *J* = 9.23 Hz), 2.95 (m, 2H), 2.25 (t, 12H, *J* = 6.46 Hz), 2.10 (d, 7H, *J* = 2.77 Hz), 2.00 (d, 3H, *J* = 6.76 Hz), 1.86 (d, 6H, *J* = 5.5 Hz), 1.00 (d, 3H, *J* = 6.13 Hz); Maldi mass: 1025 (M + Na⁺).

Per butyrate verbascoside (2b)

(Yield 41%); Colourless solid; mp: 145 °C; ¹H NMR (CDCl₃, 500 MHz): δ 6.40 (d, 1H, *J* = 16 Hz), 7.00 (s, 1H), 7.15 (s, 2H), 7.25 (d, 1H, *J* = 10 Hz), 7.34 (s, 1H), 7.46 (d, 2H, *J* = 8.5 Hz), 7.70 (d, 1H, *J* = 13.5 Hz), 5.33 (t, 1H, *J* = 3.5 Hz), 5.36 (m, 3H), 5.00 (t, 1H), 4.95 (s, 1H), 4.57 (d, 1H, *J* = 8.0 Hz), 4.48 (d, 3H, *J* = 5.5 Hz), 4.10 (d, 1H, *J* = 5.6 Hz), 4.00 (t, 1H, *J* = 4.5 Hz), 3.92 (m, 1H), 3.70 (d, 2H, *J* = 8.5 Hz), 2.90 (t, 2H, *J* = 6.5 Hz), 2.65 (m, 6H), 2.42 (d, 6H, *J* = 6.5 Hz), 2.32 (m, 4H), 2.20 (m, 3H), 1.85 (m, 10H), 1.70 (d, 8H, *J* = 3.5 Hz), 1.61 (m, 5H), 1.30 (m, 2H), 0.95 (m, 19H); Maldi mass: 1224 (M+ Na⁺).

Compound Characterization S2

3.4.3. Verbascoside 4'-butyrate (3a)

Colourless solid; mp: (158 °C); [α]^D₂₅ -90.0 (c = 1.0, CH₃OH); IR (KBr, cm⁻¹): 511, 561, 807, 857, 1036, 1090, 1114, 1157, 1260, 1375, 1446, 1602, 3380; ¹H NMR (500 MHz, DMSO): δ 7.50 (d, *J* = 15.8 Hz, 1H), 7.26 (d, *J* = 8.51 Hz, 1H), 7.19 (s, 1H), 7.0 (d, *J* = 8.19 Hz, 1H), 6.82 (d, *J* = 8.07 Hz, 1H), 6.58 (d, *J* = 7.8 Hz, 1H), 6.47 (d, *J* = 8.07 Hz, 1H), 6.25 (d, *J* = 15.7 Hz, 1H), 5.09 (s, 1H), 4.75 (s, 1H), 4.28 (d, *J* = 8.1 Hz, 1H), 3.97 (m, 1H), 3.81 (s, 1H), 3.71 (t, *J* = 5.54 Hz, 1H), 3.63 (dd, *J*_{1,2} = 8.1, *J*_{2,3} = 4.5 Hz, 1H), 3.51 (t, *J* = 10.2 Hz, 1H), 3.49 3.35 (m, 2H), 3.28 (t, *J* = 5.72 Hz, 2H), 3.20 (submerged with solvent peak, 14H), 2.8 (t, *J* = 6.32 Hz, 1H), 2.5 (q, *J* = 7.3 Hz, 1H), 2.3 (t, *J* = 2.88 Hz, 1H), 2.0 (d, *J* = 7.32 Hz, 1H), 1.7 (dd, *J* = 7.34, 3.45 Hz, 1H), 1.2 (s, 3H), 1.0 (m, 2H), 0.8 (m, 2H); ¹³C (500 MHz, CD₃OD): 14, 19, 19.5, 30, 35, 49, 54, 61, 70, 72, 74, 75, 78, 81, 102, 104, 114, 115, 117, 118, 121, 122, 124, 132, 145, 146, 165, 172; Maldi mass: (M + Na⁺) 717; Anal. Calcd for C₃₂H₄₂O₁₆: C, 57.06; H, 6.09. Found: C, 57.13; H, 6.03.

3.4.4. Verbascoside 4'', 4'-dibutyrate (**4a**)

As colourless solid; mp: 134 °C; $[\alpha]_{25}^D$ -42.0; IR (KBr, cm^{-1}): 521, 533, 758, 812, 1035, 1260, 1382, 1448, 1464, 1518, 1606, 1704, 3377; ^1H NMR (500 MHz, DMSO): δ 7.56 (d, $J = 7.33$ Hz, 1H), 6.82 (d, $J = 8.4$ Hz, 1H), 6.65 (d, $J = 7.9$ Hz, 1H), 6.25 (d, $J = 15.7$ Hz, 1H), 5.09 (s, 1H), 4.75 (d, $J = 9.9$ Hz, 1H), 4.28 (d, $J = 7.8$ Hz, 1H), 4.01 (d, $J = 7.4$ Hz, 1H), 3.81 (s, 1H), 3.72 (m, $J = 9.4$ Hz, 1H), 3.52 (t, $J = 6.03$ Hz, 1H), 3.45 (t, $J = 5.58$ Hz, 1H), 3.41 (s, 3H), 3.31 (d, $J = 8.4$ Hz, 2H), 3.21 (m, $J = 8.41$ Hz, 7H), 2.47 (q, $J = 3.07$ Hz, 3H), 1.66 (q, $J = 3.07$ Hz, 3H), 1.19 (s, 3H), 0.97 (m, 3H); ^{13}C (500 MHz, DMSO): δ 14, 19, 19.5, 58, 62, 70, 71, 75, 76, 77, 78, 79, 82, 103, 112, 113, 115, 120, 121, 125, 133, 138, 140, 157, 168, 172, 175; Maldi mass: (M + Na) 789; Anal. Calcd for $\text{C}_{37}\text{H}_{48}\text{O}_7$: C, 58.11; H, 6.33. Found: C, 58.08; H, 6.34.

3.4.5. Verbascoside 4''-octanoate (**3b**)

Colourless white solid; mp: 162 °C ; $[\alpha]_{25}^D$ -35.0 (c = 1.0, CHCl_3); IR (KBr, cm^{-1}): 476, 560, 854, 960, 1088, 1114, 1150, 1270, 1300, 1375, 1434, 1517, 1607, 1662, 1703, 3384; ^1H NMR (500 MHz, DMSO): δ 7.55 (d, $J = 15.85$ Hz, 1H), 7.47 (t, $J = 9.24$ Hz, 2H), 7.01 (d, $J = 8.26$ Hz, 1H), 6.75 (t, $J = 7.64$ Hz, 1H), 6.45 (d, $J = 7.95$ Hz, 2H), 6.39 (d, $J = 15.84$ Hz, 1H), 5.02 (s, 1H), 4.73 (s, 1H), 4.49 (t, $J = 7.61$ Hz, 1H), 3.9 (dd, $J = 6.96, 3.23$ Hz, 1H), 3.75 (m, 1H), 3.70 (dd, $J = 15.8, 6.78$ Hz, 2H), 3.31 (d, $J = 3.62$ Hz, 2H), 3.2 (dd, $J = 8.05, 4.50$ Hz, 1H), 3.19 (dd, 8.4 Hz, 1H), 3.1 (dd, $J = 9.25, 4.56$ Hz, 5H), 2.70 (t, $J = 7.26$ Hz, 2H), 1.70 (t, $J = 6.48$ Hz, 2H), 1.43 (submerged with solvent peak, 7H), 0.97 (q, 6.45 Hz, 3H); ^{13}C (500 MHz, DMSO): δ 17.5, 19.5, 23, 25, 29, 29.5, 30, 32, 37, 39, 40, 61, 69, 71, 73, 75, 80, 101, 103, 115, 116, 117, 120, 124, 129, 139, 143, 145, 165, 171; Maldi Mass: (M + Na) 773; Anal. Calcd for $\text{C}_{37}\text{H}_{50}\text{O}_{16}$: C, 59.19; H, 6.71. Found: C, 59.13; H, 6.65.

3.4.6. Verbascoside 4'', 4'-dioctanoate (**4b**)

As colourless solid ; mp: 176 °C; $[\alpha]_{25}^D$ -80.0 (1.0, CHCl_3); IR (KBr, cm^{-1}): 511, 545, 750, 807 , 857, 1036, 1078, 1125, 1145, 1200, 1350, 1145, 1530, 1656, 1710, 3380; ^1H NMR (500 MHz, DMSO): 7.57 (d, $J = 15.83$ Hz, 1H), 7.40 (t, $J = 8.5$ Hz, 2H) , 6.96 (d, $J = 9.6$ Hz, 1H), 6.8 (d, $J = 7.06$ Hz, 1H), 6.71 (d, $J = 7.95$ Hz, 2H), 6.39 (d, $J = 15.84$ Hz, 1H), 5.02 (s, 1H), 4.73 (t, $J = 9.5$ Hz, 1H), 4.36 (t, $J = 7.61$ Hz, 1H), 3.9 (m, $J = 8.75$ Hz, 1H), 3.66 (m, $J = 10.85$ Hz, 3H), 3.33

(dd, $J = 6.2, 2.3$ Hz, 2H), 3.29 (dd, $J = 7.95$ Hz, 1H), 3.2 (dd, $J = 6.17$ Hz, 1H), 3.11 (dd, $J = 3.30, 1.24$ Hz, 1H), 2.73 (dd, $J = 7.26, 5.61$ Hz, 3H), 2.79 (t, $J = 6.45$ Hz, 3H), 2.55 (t, $J = 7.07$ Hz, 1H), 2.51 (d, $J = 1.32$ Hz, 9H), 1.62 (t, $J = 7.06$ Hz, 6H), 1.36 (s, 3H), 1.24 (submerged with solvent peak), 0.96 (q, $J = 6.45$ Hz, 3H), 0.86 (t, $J = 6.48$ Hz, 3H); ^{13}C (500 MHz, DMSO): δ 14, 19, 23, 25, 29, 32, 29, 30, 31, 40, 63, 70, 71, 72, 73, 75, 80, 103, 104, 117, 120, 124, 125, 128, 137, 138, 139, 145, 148, 149, 165, 170, 173; Maldi mass: (M + Na) 899; Anal. Calcd for $\text{C}_{45}\text{H}_{64}\text{O}_{17}$: C, 61.62; H, 7.36. Found: C, 61.60; H, 7.30.

3.4.7. Verbascoside 4''-myristylate (3c)

Colourless solid; mp: 151°C; $[\alpha]_{25}^{\text{D}} -25.0$ (c 1.0, CHCl_3); IR (KBr, cm^{-1}): 510, 521, 554, 786, 806, 845, 1042, 1065, 1095, 1116, 1145, 1250, 1278, 1354, 1670, 1710, 3345; ^1H NMR (500 MHz, DMSO): δ 7.65 (d, $J = 15.83$ Hz, 1H), 7.52 (t, $J = 8.45$ Hz, 2H), 7.01 (d, $J = 8.2$ Hz, 1H), 6.64 (d, $J = 7.65$ Hz, 2H), 6.50 (d, $J = 7.95$ Hz, 1H), 6.39 (d, $J = 15.84$ Hz, 1H), 5.02 (s, 1H), 4.73 (t, $J = 9.5$ Hz, 1H), 4.36 (t, $J = 7.61$ Hz, 1H), 3.9 (dd, $J = 6.96$ Hz, 1H), 3.31 (d, $J = 3.6$ Hz, 2H), 3.29 (m, 3H), 3.19 (dd, $J = 8.4, 5.4$ Hz, 2H), 3.1 (dd, $J = 9.25, 3.45$ Hz, 2H), 2.73 (m, 3H), 2.55 (t, $J = 7.07$ Hz, 3H), 2.51 (d, $J = 1.32$ Hz, 3H), 1.62 (t, $J = 7.06$ Hz, 2H), 1.24 (submerged with solvent Peak, 17H), 0.96 (q, $J = 6.45$ Hz, 3H), 0.86 (t, $J = 6.48$ Hz, 3H); ^{13}C (500 MHz, DMSO): δ 17.5, 19, 23, 25, 29.5, 30, 32.5, 34, 36, 37, 40, 61, 69, 71, 72, 75, 80, 102, 103, 116, 117, 120, 144, 146, 163, 165, 172; Maldi mass: 858 (M + Na⁺); Anal. Calcd for $\text{C}_{43}\text{H}_{62}\text{O}_{16}$: C, 61.86; H, 7.48. Found: C, 61.83; H, 7.43.

3.4.8. Verbascoside 4'', 4'-dimyristylate (4c)

As colourless solid; mp: 143°C; $[\alpha]_{25}^{\text{D}} -65.0$ (c 1.0, CHCl_3); IR : (KBr, cm^{-1}): 501, 630, 650, 757, 820, 1039, 1419, 1642, 1719, 2871, 2936, 3399; ^1H NMR (500 MHz, DMSO): δ 7.53 (d, $J = 15.08$ Hz, 1H), 7.28 (t, $J = 8.5$ Hz, 1H), 7.20 (s, 1H), 6.84 (d, $J = 8.4$ Hz, 1H), 6.79 (d, $J = 7.95$ Hz, 1H), 6.75 (d, $J = 7.8$ Hz, 1H), 6.65 (d, $J = 8.17$, 1H), 6.25 (d, $J = 15.84$ Hz, 1H), 5.02 (s, 1H), 4.73 (s, 1H), 4.28 (t, $J = 7.61$ Hz, 1H), 3.9 (m, 1H), 3.82 (s, 1H), 3.72 (m, 1H), 3.54 (d, $J = 4.92$ Hz, 1H), 3.43 (d, $J = 2.84$ Hz, 4H), 3.21 (m, 6H), 2.78 (d, $J = 8.56$ Hz, 1H), 2.5 (m, 3H), 1.62 (t, $J = 7.06$ Hz, 3H), 1.34 (s, 3H), 1.19 (submerged with solvent peak), 0.96 (d, $J = 6.45$ Hz, 14H), 0.86 (t, $J = 6.48$ Hz, 8H); ^{13}C (500 MHz, DMSO): δ 14, 19, 23, 30, 31, 35, 37, 46, 58, 62, 70, 71, 74, 75, 76, 79, 80, 82, 103, 112, 115, 117, 118, 120, 122, 130, 134, 146, 147, 148, 153, 162, 168,

171, 173; Maldi mass: (M + Na) 1045; Anal. Calcd for C₅₈H₉₀O₁₇: C, 65.76; H, 8.56. Found: C, 65.73; H, 8.53.

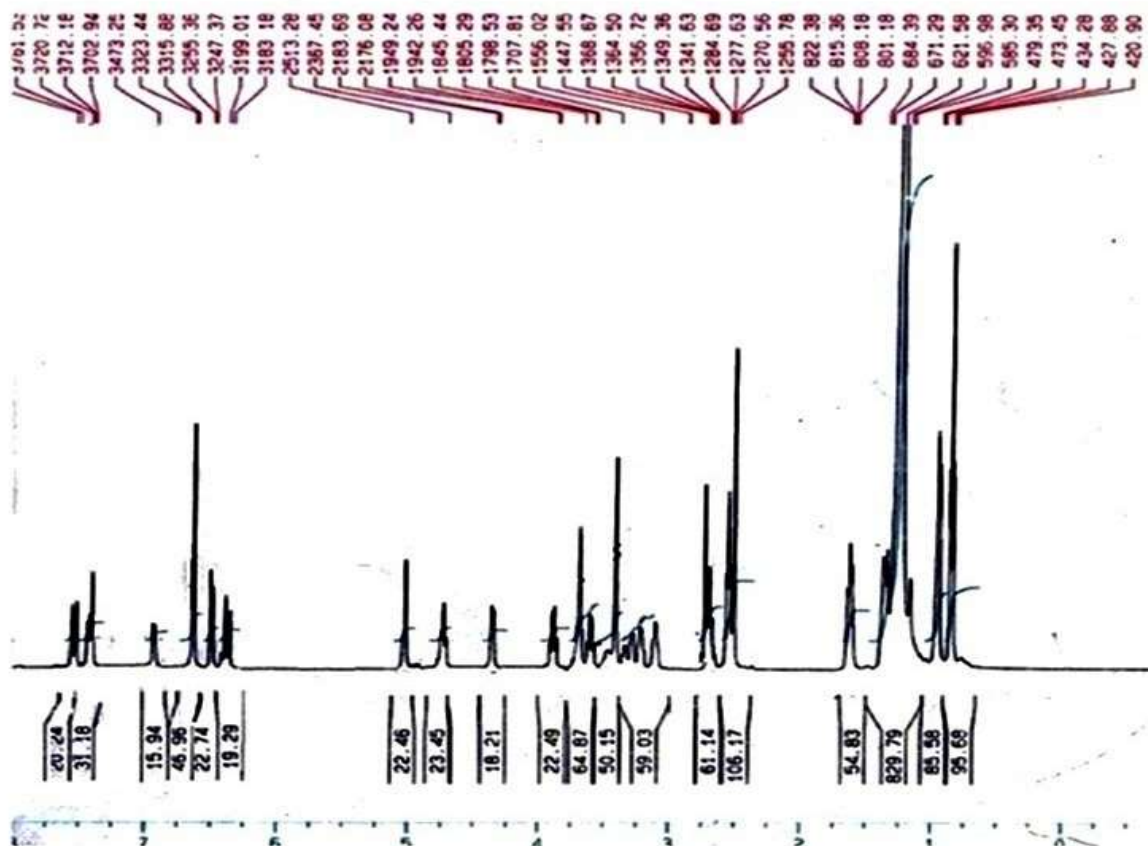
3.4.9. *Verbascoside 4''-palmitate (3d)*

As colourless solid; mp: 183° C; [α]_D²⁵ -33.3 (c 1.0, CHCl₃); IR (KBr, cm⁻¹): 501, 523, 545, 653, 780, 790, 830, 1043, 1097, 1132, 1178, 1267, 1368, 1724, 3330; ¹H NMR (500 MHz, DMSO): δ 7.65 (d, *J* = 15.83 Hz, 1H), 7.47 (t, *J* = 9.24 Hz, 2H), 7.01 (d, *J* = 8.26 Hz, 1H), 6.64 (t, *J* = 7.56 Hz, 2H), 6.48 (d, *J* = 7.8 Hz, 1H), 6.25 (d, *J* = 15.79 Hz, 1H), 5.02 (s, 1H), 4.73 (t, *J* = 9.5 Hz, 1H), 4.49 (t, *J* = 7.51 Hz, 1H), 3.9 (dd, *J* = 6.96 Hz, 1H), 3.75-3.70 (m, 3H), 3.31 (d, *J* = 3.62 Hz, 2H), 3.29-3.19 (m, 5H), 2.8 (t, *J* = 7.47 Hz, 3H), 1.70 (t, *J* = 6.48 Hz, 2H), 1.43 (submerged with solvent peak, 27H), 1.03 (d, 6.45 Hz, 3H), 0.92 (t, *J* = 6.19 Hz, 3H); ¹³C (500 MHz, DMSO): δ 15, 19, 25, 28, 32, 35, 38, 39, 50, 64, 71, 72, 73, 75, 76, 82, 104, 105, 116, 117, 119, 120, 122, 124, 125, 129, 130, 134, 142, 146, 147, 149, 165, 172; Maldi mass: (M + Na⁺) 872; Calcd for C₄₄H₆₄O₁₆: C, 62.25; H, 7.60. Found: C, 62.21; H, 7.54.

3.4.10. *Verbascoside 4'', 4'-dipalmitate (4d)*

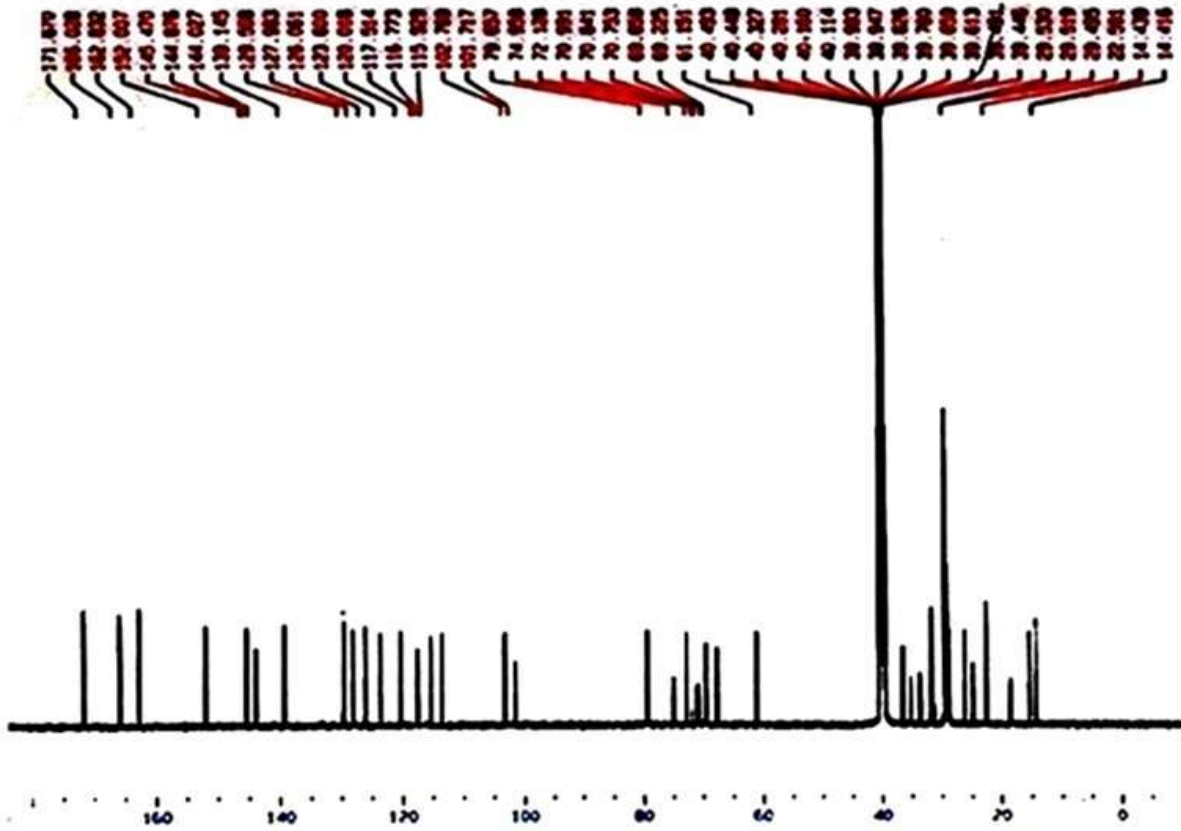
As colourless solid; mp: 188 °C; [α]_D²⁵ -54.0 (c 1.0, CDCl₃); IR (KBr, cm⁻¹): 521, 590, 630, 675, 750, 845, 1035, 1420, 1645, 1739, 2834, 3345; ¹H NMR (500 MHz, DMSO): δ 7.53 (d, *J* = 15.08 Hz, 1H), 7.28 (t, *J* = 8.5 Hz, 1H), 7.2 (s, 1H), 6.84 (d, *J* = 8.4 Hz, 1H), 6.79 (d, *J* = 7.95 Hz, 1H), 6.75 (d, *J* = 7.8 Hz, 1H), 6.65 (d, *J* = 8.17 Hz, 1H), 6.25 (d, *J* = 15.84 Hz, 1H), 5.02 (s, 1H), 4.73 (s, 1H), 4.28 (t, *J* = 7.61 Hz, 1H), 3.9 (m, *J* = 8.75 Hz, 1H), 3.82 (s, 1H), 3.72 (m, 1H), 3.54 (d, *J* = 4.92 Hz, 2H), 3.43 (d, *J* = 2.84 Hz, 4H), 3.21 (m, 6H), 2.78 (d, *J* = 8.56 Hz, 1H), 2.5 (m, 3H), 1.62 (t, *J* = 7.06 Hz, 3H), 1.34 (s, 3H), 1.19 (submerged with solvent peak), 0.96 (d, *J* = 6.45 Hz, 14H), 0.86 (t, *J* = 6.48 Hz, 8H); ¹³C (500 MHz, DMSO): δ 14, 19, 23, 30, 31, 35, 37, 46, 58, 62, 70, 71, 74, 75, 76, 79, 80, 82, 103, 112, 115, 117, 118, 120, 122, 130, 139, 146, 147, 148, 153, 165, 170, 173; Maldi mass: (M + Na) 1069; Anal. Calcd for C₆₀H₉₄O₁₇: C, 66.76; H, 8.71. Found: C, 66.73; H, 8.53.

ACT-L1D



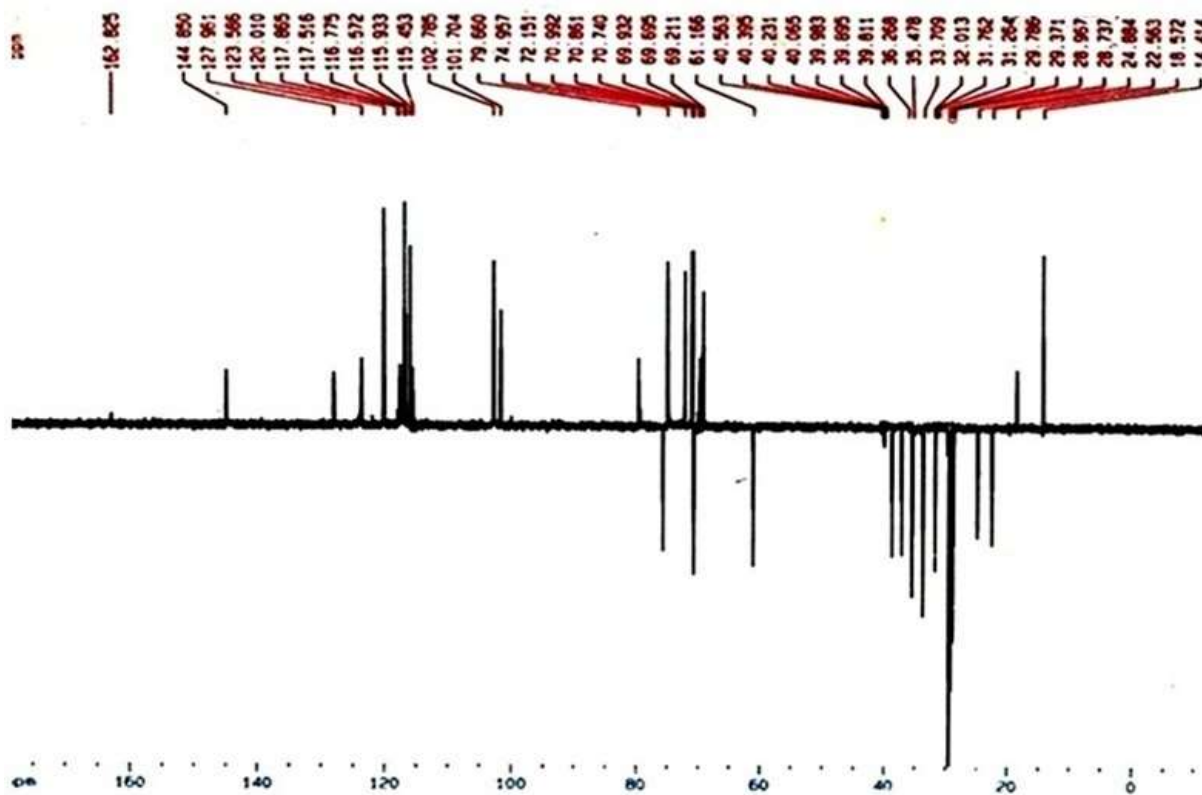
¹H NMR of Verbascoside 4''-palmitate (3d)

ACT-6.10



^{13}C NMR of verbascoside 4''-palmitate

ACT-L1D



DEPT of verbascoside 4''-palmitate (3d)



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Yellst Peak SN	1262.6423
Yellst Peak Mass	901.3946
Yellst Peak Resolution	10429.3340
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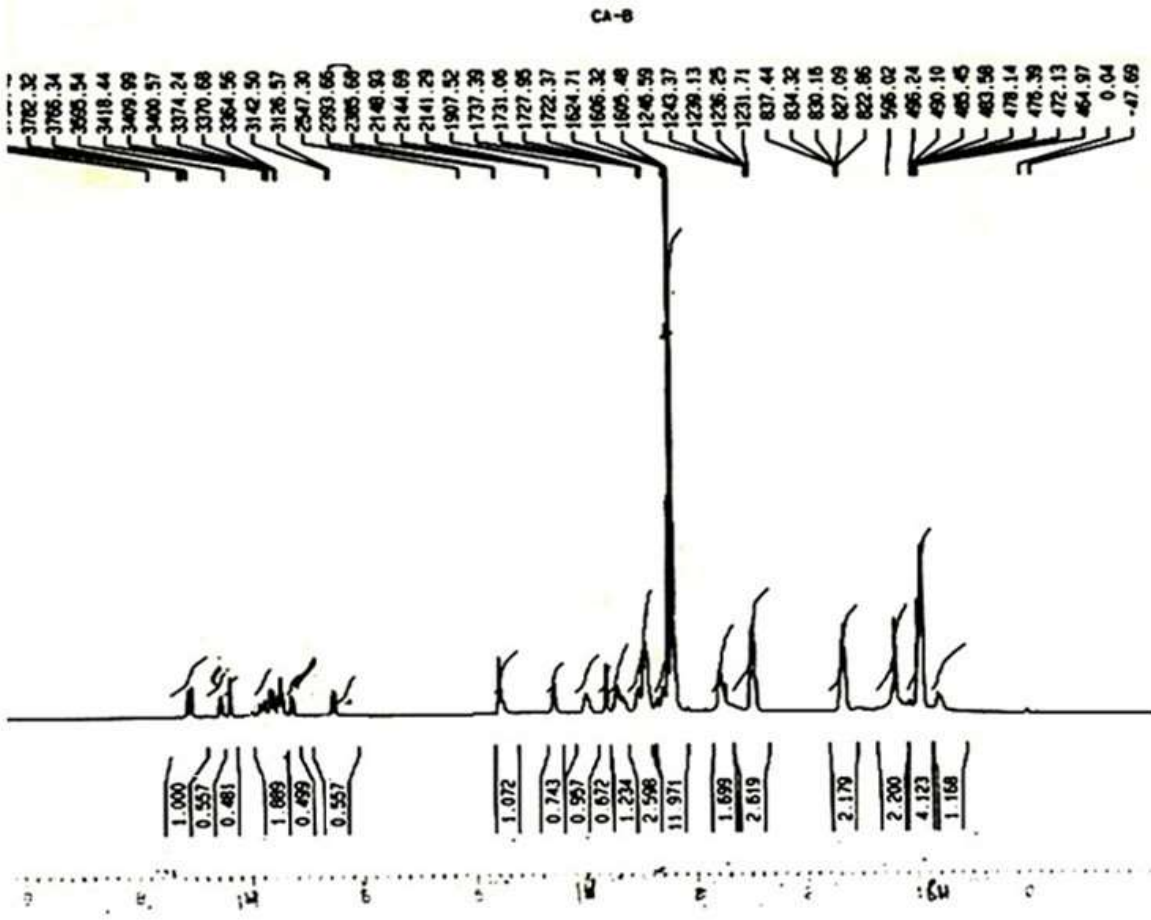
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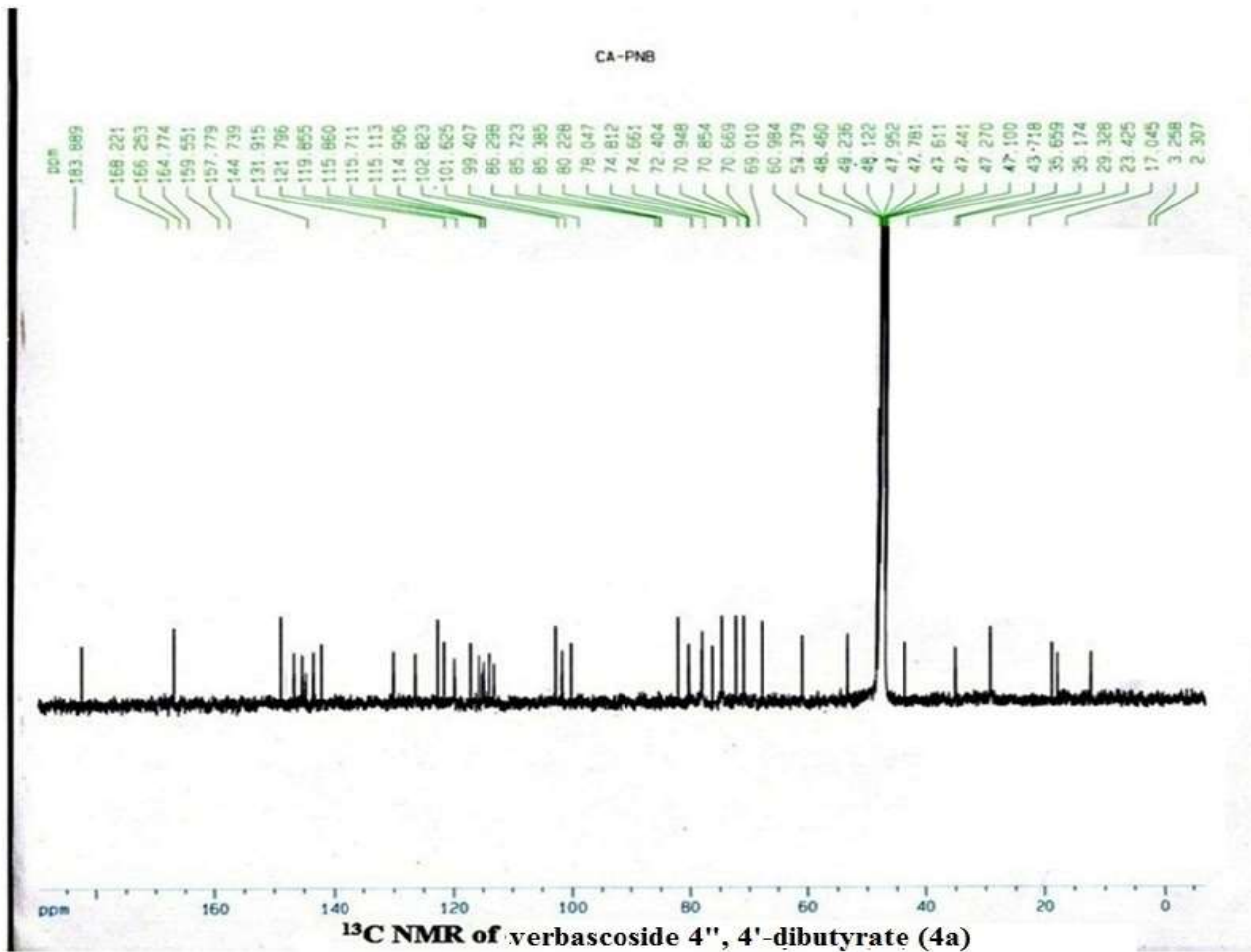
Page 1

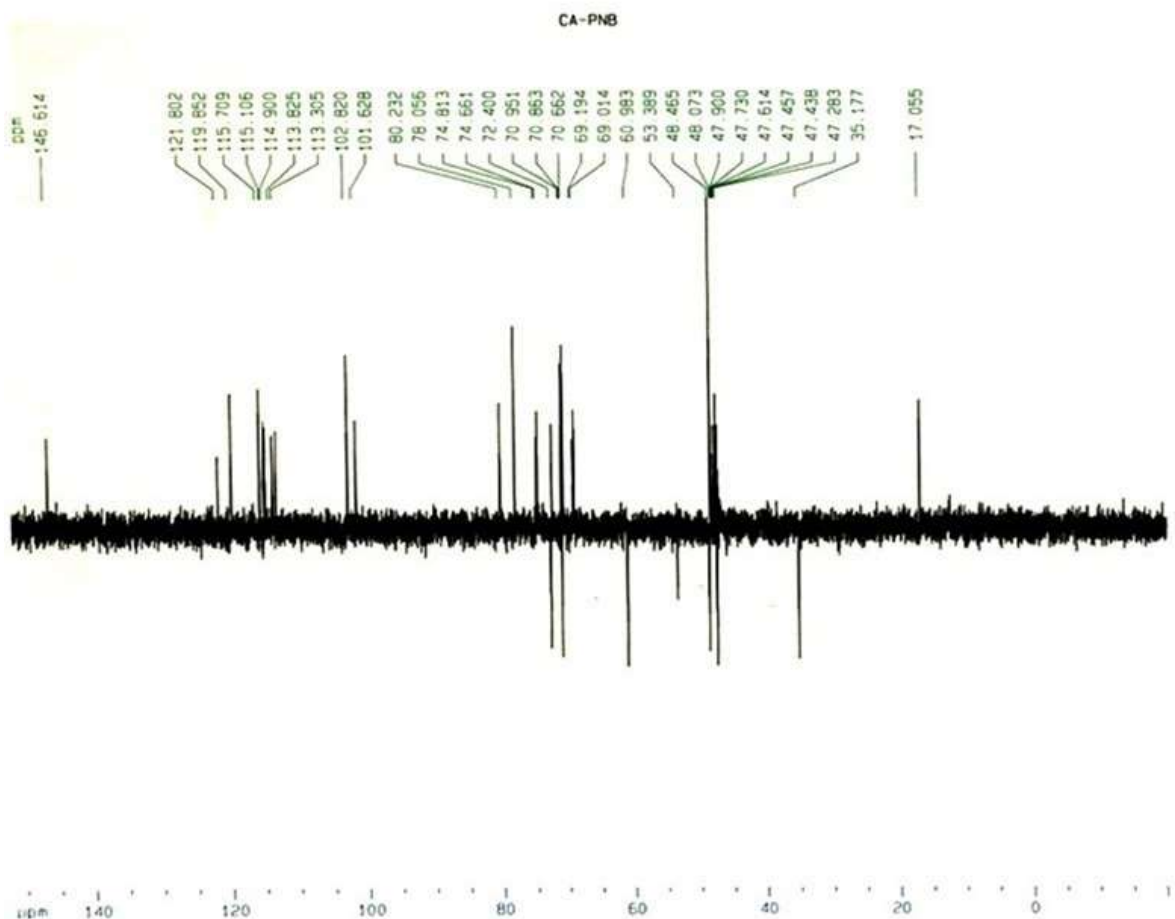
3/12/2009 12:22:02 PM

MALDI-MS of verbascoside 4''-palmitate(3d)



1H NMR of verbascoside 4'', 4'-dibutyrate (4a)

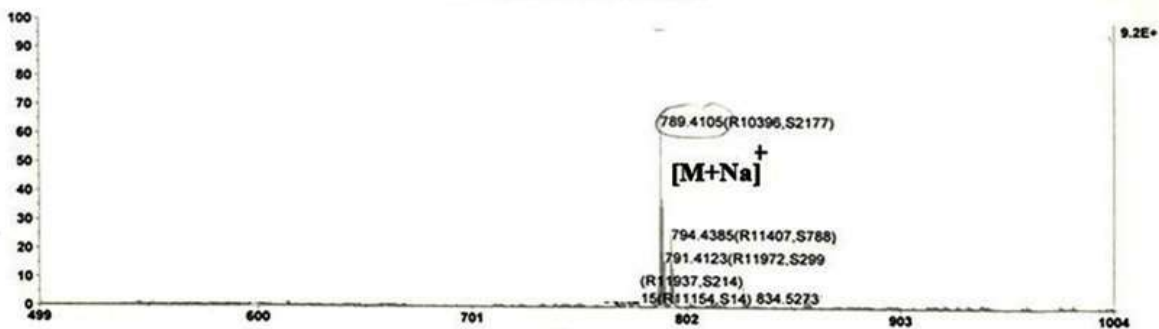




Dept of verbascoside 4'',4'-dibutyrate (4a)

Spectrum Report

Final - Shots 400 - sds pep; Label N3



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Acquisition
Method Name MS Reflector Positive

sds/sds pep Label N3 Run # 8

Page 1

Maldi MS of verbascoside 4'',4'-dibutyrate (4a)