SUPPLEMENTARY MATERIAL

New lipoxygenase and cholinesterase inhibitory sphingolipids from *Carthamus oxyacantha*

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Two new sphingolipids: oxyacanthin A [(2*S*,3*S*,4*R*)-2-{[(2*R*,5*E*)-2-hydroxyoctadec-5-enoyl]amino}hexaeicosane-1,3,4-triol; **1**] and B [(2*S*,3*S*,4*R*)-2-{[(2*R*,5*E*)-2-hydroxyoctadec-5-enoyl]amino}hexaeicosane-1,3,4-triol-1-*O*- β -D-glucopyranoside; **2**], together with 1-octacosanol, β -sitosterol, β -sitosterol 3-*O*- β -D-glucopyranoside and luteolin 7-*O*- β -glucopyranoside were isolated from the methanolic extract of the whole plant of *Carthamus oxyacantha*. Their structures were elucidated using ¹H and ¹³C NMR spectra and 2D NMR analyses (HMQC, HMBC and COSY) in combination with mass spectrometry (EI-MS, HR-EI-MS, FAB-MS and HR-FAB-MS) experiments and in comparison with the literature data of the related compounds. Both the compounds **1,2** showed inhibitory potential against lipoxygenase (LOX) in a concentration dependent manner with IC₅₀ values 83.3 $\pm$ 1.3 and 245.7 $\pm$ 1.1 μ M, whereas compound **2** showed inhibition against enzymes acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) with IC₅₀ values 65.3 $\pm$ 0.1 and 93.6 $\pm$ 0.1 μ M, respectively.

Keywords: *Carthamus oxyacantha*; sphingolipids; secondary metabolites; lipoxygenase inhibition; cholinesterase inhibition

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S1: EI-MS Spectrum of Compound **1** (oxyacanthin A)

File Name: M6-2653
Sample: M. Dilshad/Dr. M. Saleem, Chem Deptt. The Islamia University of Bahawalpur
Instrument: JEOL JMS600
Inlet: My Inlet

Date Run: 21-11-2012
Ionization mode: HRMS⁺

Time Run: 10:45:12

Run By: Lab 102
Printed by: Lab 102

Mass	Relative Intensity	Theoretical Delta		Delta	RDB	Composition
		Mass	[ppm]	[mmu]		
709.65673	3.9809	709.65841	3.1	0.7	5.0	C ₄₄ H ₆₇ NO ₅
692.65701	4.4072	692.65568	3.4	0.9	5.5	C ₄₄ H ₆₉ NO ₄
677.63401	9.1440	677.63222	7.4	1.6	10.0	C ₄₃ H ₆₂ NO ₄
647.65705	45.67541	647.65568	20.3	4.3	17.0	C ₄₃ H ₇₀ NO ₄
631.66115	12.0298	631.66077	3.0	0.9	5.7	C ₄₃ H ₇₁ NO ₅
617.64672	8.76542	617.64513	2.7	3.2	4.9	C ₄₈ H ₇₃ NO ₅
603.63001	4.97654	603.62949	17.6	3.7	0.5	C ₃₅ H ₇₄ NO ₅
589.61545	2.33151	589.61385	3.5	0.7	9.0	C ₃₈ H ₇₂ NO ₅
561.58589	2.76854	561.58257	1.3	1.4	3.5	C ₃₈ H ₆₀ NO ₅
547.56861	2.77659	547.56693	1.2	1.3	3.4	C ₃₅ H ₆₀ NO ₅
533.1345	2.12452	533.55129	1.2	1.4	3.5	C ₃₄ H ₆₀ NO ₅
519.53641	2.43541	519.53565	1.3	1.3	3.6	C ₃₃ H ₆₂ NO ₅
505.52254	2.67439	505.52001	1.2	1.3	3.4	C ₃₂ H ₆₀ NO ₅
491.50623	2.56723	491.50437	1.2	1.3	3.3	C ₃₃ H ₅₈ NO ₅
477.48999	3.98543	477.48873	1.4	1.3	3.1	C ₃₈ H ₅₆ NO ₅
463.47556	4.01267	463.47309	1.6	1.4	3.9	C ₂₈ H ₅₄ NO ₅
453.38345	8.56319	453.38180	1.9	1.6	4.1	C ₂₇ H ₅₃ NO ₄
439.36789	25.4926	439.36166	2.9	1.9	8.0	C ₂₈ H ₄₈ NO ₄
425.35234	16.5952	425.53052	2.2	1.6	7.5	C ₂₅ H ₄₃ NO ₄

S2: HREI-MS Spectrum of Compound 1 oxycanthin A

S3: ¹H-NMR (600 MHz, CDCl₃) Spectrum of Compound **1** (oxycanthin A)

S4: ¹H-NMR Spectrum of Compound **1** (oxycanthin A) (From 5.23 to 3.18 ppm)

S5: ¹H-NMR Spectrum of Compound **1** (oxycanthin A) (From 1.83 to 0.69 ppm)

S7: ^{13}C -NMR + DEPT Spectrum of Compound **1** (oxyacanthin A)

S8: COSY (600 MHz) Spectrum of Compound **1** (oxyacanthin A)

S9: COSY Spectrum of Compound **1** (oxyacanthin A) (From 5.5 to 0.80 ppm)

S10: HSQC (600 MHz) Spectrum of Compound 1 (oxyacanthin A)

S11: HSQC Spectrum of Compound **1** (oxycanthin A) (From 50 to 130 ppm)

S12: HMBC Spectrum of Compound **1** (oxycanthin A)

S13: NOESY Spectrum of Compound **1** (oxycanthin A)

File Name: M6-2652

Date Run: 25-11-2012

Time Run: 12:14:02

Sample: M. Dilshad/Dr. M. Saleem, Chem Deptt. The Islamia University of Bahawalpur

Instrument: JEOL JMS-500

Run By: Lab 101

Inlet: My Inlet

Ionization mode: HRFABMS [-ve mode]

printed by: Lab 101

Mass	Relative Intensity	Theoretical Mass	Composition
870.70485	8.67342	870.70342	C ₅₀ H ₉₆ NO ₁₀
870.71859	8.67342	870.71600	C ₅₁ H ₉₈ O ₁₀
870.74140	8.67342	870.73980	C ₅₁ H ₁₀₀ NO ₉

S15: HRFAB-MS Spectrum of Compound 2 (Oxyacanthin B)

S16: ^1H -NMR (500 MHz, CD_3OD) Spectrum of Compound **1** (oxycanthin B)

S17: ^1H -NMR Spectrum of Compound **2** (oxyacanthin B) (From 4.30 to 3.40 ppm)

S18: ^1H -NMR Spectrum of Compound **2** (oxyacanthin B) (From 3.52 to 3.15 ppm)

S19: ^{13}C -NMR + DEPT (125 MHz, CD_3OD) Spectrum of Compound **2** (oxyacanthin B)

S20: ^{13}C -NMR + DEPT Spectrum of Compound **2** (oxyacanthin B)

S21: COSY (500 MHz) Spectrum of Compound 2 (oxyacanthin B)

S22: HSQC (500 MHz) Spectrum of Compound **2** (oxyacanthin B)

S23: HSQC Spectrum of Compound **2** (Oxyacanthin B) (From 55 to 90 ppm)

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I.U.BWP/

S24: HMBC (500 MHz) Spectrum of Compound **2** (oxyacanthin B)

Table 1. ^1H and ^{13}C NMR spectral data, HMBC and COSY correlations of **1** ($\text{CDCl}_3+\text{CD}_3\text{OD}$, 500, 125 MHz)

Position	δ_{H} , (J in Hz)	δ_{C}	HMBC (H $\rightarrow$ C)	COSY (H $\rightarrow$ H)
1	3.63 (1H, dd, 11.5, 3.8) 3.57 (1H, dd, 11.5, 5.0)	60.6	2,3	H-1/H-2
2	3.91 (1H, m)	51.5	1,3,4,1'	H-2/H-1,3
3	3.46 (1H, dd, 4.1, 3.7)	75.1	1,2,4,5	H-3/H-2,4
4	3.38 (1H, m)	72.7	2,3,5,6	H-4/H-3,5
5,3'	1.61 (4H, m)	33.7, 35.7	3,4,6,1',2',4'	H-5,3'/H-4,6,2',4'
6-25,8'-17'	1.08 (60H, br s)	29.1-30.2	5,24,7',18'	H-6,25/H-5,26 H-8',17'/H-9',17'
26,18'	0.71 (6H, t, 6.5)	14.5	24,25,16',17'	H-26,18'/H-25,17'
NH	7.80 (1H, d, 8.8)	-	1,2,3,1',2'	N-H/H-1,H-2
1'	-	173.2	-	-
2'	3.82 (1H, dd, 6.6, 3.4)	72.9	1',3'	H-2'/H-3'
4',7'	1.54 (4H, m)	35.5, 33.8	6',5',9'	H-4',7'/H-3',5',6',8'
5',6'	5.32 (2H, br m)	131.4	4',7'	H-5',6'/H-4',6',7'

S25: Table 1. ^1H and ^{13}C NMR spectral data, HMBC and COSY correlations of **1** ($\text{CDCl}_3+\text{CD}_3\text{OD}$, 500, 125 MHz)

Table 2. ^1H and ^{13}C NMR spectral data, HMBC and COSY correlations of **2** (CD_3OD , 500, 125 MHz)

Position	δ_{H} , (J in Hz)	δ_{C}	HMBC (H $\rightarrow$ C)	COSY (H $\rightarrow$ H)
1	4.04 (1H, dd, 11.6, 3.9) 3.87 (1H, dd, 11.6, 5.1)	69.9	2,3,1''	H-1/H-2
2	4.24 (1H, m)	51.6	1,3,4,1'	H-2/H-1,3
3	3.81 (1H, dd, 4.2, 3.9)	75.5	1,2,4,5	H-3/H-2,4
4	3.60 (1H, m)	73.0	2,3,5,6	H-4/H-3,5
5,3'	1.61 (4H, m)	33.7, 35.7	3,4,6,1',2',4'	H-5,3'/H-4,6,2',4'
6-25, 8'-17'	1.08 (60H, br s)	29.1-30.2	5,24,7',18'	H-6,25/H-5,26 H-8',17'/H-7',18'
26, 18'	0.71 (6H, t, 6.4)	14.5	24,25,16',17'	H-26,18'/H-25,17'
NH	7.82 (1H, d, 8.7)	-	1,2,3,1',2'	N-H/H-2
1'	-	177.1	-	-
2'	4.06 (1H, dd, 6.4, 3.6)	72.9	1',3'	H-2'/H-3'
4',7'	1.54 (4H, m)	35.5, 33.8	6',5',9'	H-4',7'/H-3',5',6',8'
5', 6'	5.41 (2H, br m)	131.4	4',7'	H-5',6'/H-4',6',7'
1''	4.28 (1H, d, 7.5)	104.7	1,2'',3''	H-1''/H-2''
2''	3.39 (1H, m)	77.9	1'',3'',4''	H-2''/H-1'',3''
3''	3.36 (1H, m)	72.9	1'',2'',4'',5''	H-3''/H-2'',4''
4''	3.65 (1H, m)	71.5	3'',5''	H-4''/H-3'',5''
5''	3.79 (1H, m)	78.0	3'',6''	H-5''/H-4'',6''
6''	3.85 (1H, dd, 10.4, 4.5) 3.71 (1H, dd, 10.4, 2.9)	62.6	5'',4''	H-6''/H-5'',6''

S26: Table 1. ^1H and ^{13}C NMR spectral data, HMBC and COSY correlations of **2** (CD_3OD , 500, 125 MHz)

Figure 2. EI-MS fragmentation pattern of **1** and **2**

S27: Figure 2. EI-MS fragmentation pattern of **1** and **2**

S28: Inhibition (%) of LOX, AChE, BChE of compounds **1** & **2** at various concentrations. Data is mean of three independent experiments.