Supporting information

Iridoid Glycosides from *Barleria lupulina*

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Figure S1. HR-ESI MS of **1**

Single Mass Analysis

Tolerance = 10.0 PPM / DBE: min = -1.5, max = 600.0

Element prediction: Off

Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions

88 formula(e) evaluated with 2 results within limits (all results (up to 1000) for each mass)

Elements Used:

C: 0-120 H: 0-200 O: 10-20 Na: 0-1

Ki-hyun Kim, BL80, with NaCl

University of Illinois, SCS, Mass Spectrometry Lab

Qtof_47018a 9 (0.679) AM (Cen,3, 80.00, Ar,15000.0,558.36,0.70,LS 3); Sm (SG, 2x5.00)

Q-tof UE521

1: TOF MS ES+

6.09e+002

Minimum: -1.5
Maximum: 5.0 10.0 600.0

Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Formula			
499.1431	499.1428	0.3	0.6	6.5	4.1	C20	H28	O13	Na
	499.1452	-2.1	-4.2	9.5	4.8	C22	H27	O13	

Figure S2. ^1H NMR spectrum of **1** in methanol- d_4

Figure S3. ^1H - ^1H COSY spectrum of **1** in methanol- d_4

Figure S4. HSQC spectrum of **1** in methanol- d_4

Figure S5. HMBC spectrum of **1** in methanol- d_4

Figure S6. NOESY spectrum of **1** in methanol- d_4

Figure S7. HR-ESI MS of 2

Single Mass Analysis

Tolerance = 10.0 PPM / DBE: min = -1.5, max = 600.0

Element prediction: Off

Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions

94 formula(e) evaluated with 2 results within limits (all results (up to 1000) for each mass)

Elements Used:

C: 0-120 H: 0-200 O: 10-20 Na: 0-1

Ki-hyun Kim, BL78, with NaCl

University of Illinois, SCS, Mass Spectrometry Lab

Qtof_47017b 10 (0.754) AM (Cen,3, 80.00, Ar,15000.0,558.36,0.70,LS 3); Sm (SG, 2x5.00); Cm (9:11)

Q-tof UE521

1: TOF MS ES+

7.08e+002

Minimum: -1.5
Maximum: 5.0 10.0 600.0

Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Formula			
541.1525	541.1533	-0.8	-1.5	7.5	1.2	C22	H30	O14	Na
	541.1557	-3.2	-5.9	10.5	0.3	C24	H29	O14	

Figure S8. ^1H NMR spectrum of **2** in methanol- d_4

Figure S9. ^1H - ^1H COSY spectrum of **2** in methanol- d_4

Figure S10. TOCSY spectrum of **2** in methanol- d_4

Figure S11. HSQC spectrum of **2** in methanol- d_4

Figure S12. HMBC spectrum of **2** in methanol- d_4

Figure S13. NOESY spectrum of **2** in methanol- d_4

Figure S14. HR-ESI MS of **3**

Single Mass Analysis

Tolerance = 10.0 PPM / DBE: min = -1.5, max = 600.0

Element prediction: Off

Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions

780 formula(e) evaluated with 8 results within limits (all results (up to 1000) for each mass)

Elements Used:

C: 0-130 H: 0-250 N: 0-5 O: 0-13 Na: 0-1

Ki-hyun Kim, BL99

University of Illinois, SCS, Mass Spectrometry Lab

Qtof_48087 29 (2.171) AM (Cen,3, 80.00, Ar,15000.0,716.46,0.70,LS 3); Sm (SG, 2x5.00); Cm (29:30)

Q-tof UE521

1: TOF MS ES+

6.84e+002

Minimum: -1.5
Maximum: 5.0 10.0 600.0

Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Formula			
443.1167	443.1165	0.2	0.5	5.5	1.3	C17	H24	O12	Na
	443.1160	0.7	1.6	23.5	26.3	C30	H16	N2	O Na
	443.1179	-1.2	-2.7	10.5	3.3	C18	H20	N4	O8 Na
	443.1184	-1.7	-3.8	26.5	32.4	C32	H15	N2	O
	443.1190	-2.3	-5.2	8.5	1.9	C19	H23	O12	
	443.1144	2.3	5.2	22.5	19.7	C27	H15	N4	O3
	443.1131	3.6	8.1	17.5	13.8	C26	H19	O7	
	443.1203	-3.6	-8.1	13.5	5.1	C20	H19	N4	O8

Figure S15. ^1H NMR spectrum of **3** in methanol- d_4

Figure S16. ^1H - ^1H COSY spectrum of **3** in methanol- d_4

Figure S17. TOCSY spectrum of **3** in methanol- d_4

Figure S18. HSQC spectrum of **3** in methanol- d_4

Figure S19. HMBC spectrum of **3** in methanol- d_4

Figure S20. HR-ESI MS of **4**

Single Mass Analysis

Tolerance = 10.0 PPM / DBE: min = -1.5, max = 600.0

Element prediction: Off

Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions

129 formula(e) evaluated with 2 results within limits (all results (up to 1000) for each mass)

Elements Used:

C: 0-130 H: 0-250 N: 0-5 O: 11-13 Na: 0-1

Ki-hyun Kim, BL98

University of Illinois, SCS, Mass Spectrometry Lab

Qtof_48086a 31 (2.320) AM (Cen,3, 80.00, Ar,15000.0,716.46,0.70,LS 3); Sm (SG, 2x5.00); Cm (31:32)

Q-tof UE521

1: TOF MS ES+

7.11e+002

Minimum:

Maximum:

-1.5

5.0

10.0

600.0

Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Formula			
443.1164	443.1165	-0.1	-0.2	5.5	9.8	C17	H24	O12	Na
	443.1190	-2.6	-5.9	8.5	12.9	C19	H23	O12	

Figure S21. ^1H NMR spectrum of **4** in methanol- d_4

Figure S22. ^1H - ^1H COSY spectrum of **4** in methanol- d_4

Figure S23. TOCSY spectrum of **4** in methanol- d_4

Figure S24. HSQC spectrum of **4** in methanol- d_4

Figure S25. HMBC spectrum of **4** in methanol- d_4

Figure S26. LC/MS analyses of compounds **1-4** in the crude extract of the dried aerial parts of *B. lupulina*

HR-MS analysis was performed using a Waters UPLC-QTOF Xevo G2-S mass spectrometer (Waters Corporation, Milford, CT, USA)

Column: ACQUITY UPLC BEH C18, 2.1 × 100 mm, 1.7 μm, Waters; flow rate = 0.4 mL/min; Column oven temp. = 40 °C; Run time = 6.0 min; condition = 5% aqueous MeCN (+0.1% formic acid) for 0.5 min, gradient from 5% aqueous MeCN (+0.1% formic acid) to 90% aqueous MeCN (+0.1% formic acid) for the next 3.5 min, 90% aqueous MeCN (+0.1% formic acid) for the next 0.5 min, and 5% aqueous MeCN (+0.1% formic acid) for the next 1.5 min.

The molecular ion extractions of the peaks indicating the presence of **1-4** in the above crude extract of *B. lupulina* in LC/MS

The molecular ion extractions of standard compounds **1-4** in LC/MS (The standard compounds **1-4** that were used for this study were isolated from *B. lupulina*)