SUPPLEMENTARY MATERIAL

Two New Cytotoxic Furoquinoline Alkaloids Isolated from *Aegle marmelos* (Linn.) Correa

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Abstract

Two new cytotoxic furoquinoline alkaloids were isolated from the leaves of *Aegle marmelos* (Linn.) Correa; one from the total alkaloidal fraction (acid/base shake-out method) of the CHCl₃ extract, and identified as 7,8-dihydroxy-4-hydrofuroquinoline and named trivially as Aegelbine-A. The other new alkaloid isolated from the pet. ether extract and identified as 4-hydro-7-hydroxy-8-prenyloxyfuroquinoline and named trivially as Aegelbine-B, together with a known alkaloid; aegeline and a known phenolic acid; *p*-hydroxybenzoic acid. The structures of all the isolated compounds were established based on 1D and 2D NMR spectroscopy and HR-ESI/MS. The cytotoxic activity of the isolated compounds was evaluated *in vitro* against HepG-2, PC3, A549 and MCF-7 cell lines. The obtained results revealed promising activity with structure based relationship which discussed briefly.

Keywords: *Aegle marmelos* (Linn.) Correa; Rutaceae; furoquinoline alkaloids; cytotoxicity; structure–activity relationship studies.

Selected HMBC correlations are indicated by (→) and ¹H-¹H COSY correlations are indicated by bold bonds (—) for compounds (**1** & **2**).

- S1: ¹H-NMR spectrum of compound (**1**)
- S2: ¹³C-NMR spectrum of compound (**1**)
- S3: COSY spectrum of compound (**1**)
- S4: DEPT-135 spectrum of compound (**1**)
- S5: HSQC spectrum of compound (**1**)
- S6: HMBC spectrum of compound (**1**)
- S7: HR-ESI/MS spectrum of compound (**1**)

- S8: ¹H-NMR spectrum of compound (**2**)
- S9: ¹³C-NMR spectrum of compound (**2**)
- S10: COSY spectrum of compound (**2**)
- S11: DEPT-135 spectrum of compound (**2**)
- S12: HSQC spectrum of compound (**2**)
- S13: HMBC spectrum of compound (**2**)
- S14: HR-ESI/MS spectrum of compound (**2**)

S15: The Cytotoxicity data

Acquisition Time (sec)	1.9923	Comment	5 mm PABBO BB-1H/D Z-GRD Z1086180050	Date	03 Oct 2012 08:19:12
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Sweep Width (Hz)	8223.18	Temperature (degree C)	23.860	Pulse Sequence	zg30
				Spectrum Offset (Hz)	1992.2268

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Original Points Count	32768	Owner	nmr	Pulse Sequence	zgpg30
Receiver Gain	203.00	SW(cyclical) (Hz)	24038.46	Spectrum Offset (Hz)	10174.9248
Sweep Width (Hz)	24037.73	Temperature (degree C)	25.060		

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Frequency (MHz)		100.62	Nucleus	13C	Number of Transients	256	Origin	spect
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Receiver Gain		203.00	SW(cyclical) (Hz)		24038.46	Solvent	MeOD	Spectrum Offset (Hz)
Sweep Width (Hz)		24037.73	Temperature (degree C)		24.360			

mmd_17_DEPT-135_010000fid

Mass Spectrum SmartFormula Report

Analysis Info

Analysis Name D:\Data\PKHVAO 916_20121204.d
Method Standard pos low.m
Sample Name
Comment

Acquisition Date 12/4/2012 1:08:25 PM

Operator BDAL@DE
Instrument / Ser# microTOF-Q II 10205

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.3 Bar
Focus	Not active	Set Capillary	4200 V	Set Dry Heater	180 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min
Scan End	1500 m/z	Set Collision Cell RF	300.0 Vpp	Set Divert Valve	Source

Acquisition Time (sec)	1.9923	Comment	5 mm PABBO BB-1H/D Z-GRD Z108618/0050	Date	03 Oct 2012 08:29:52
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Frequency (MHz)	400.12	Nucleus	1H	Origin	spect
Original Points Count	16384	Owner	nmr	Points Count	16384
Receiver Gain	203.00	SW(cyclical) (Hz)	8223.68	Solvent	MeOD
Sweep Width (Hz)	8223.18	Temperature (degree C)	23.960	Pulse Sequence	zg30
				Spectrum Offset (Hz)	3571.1887

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Acquisition Time (sec)	1.3631	Comment	5 mm PABBO BB-1H/D Z-GRD Z108618/0050	Date	30 Oct 2012 01:18:56
Date Stamp	30 Oct 2012 01:18:56				
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Frequency (MHz)	100.62	Nucleus	13C	Number of Transients	8192
Original Points Count	32768	Owner	nmr	Points Count	32768
Receiver Gain	203.00	SW(cyclical) (Hz)	24038.46	Solvent	CHLOROFORM-d
Sweep Width (Hz)	24037.73	Temperature (degree C)	24.960	Pulse Sequence	zgpg30
				Spectrum Offset (Hz)	10056.7568

mmd_18_13C_010000fid.esp

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Date Stamp 30 Oct 2012 01:31:44					
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Frequency (MHz)	100.62	Nucleus	13C	Number of Transients	256 spect
Original Points Count	32768	Owner	nmr	Points Count	32768 dept135
Receiver Gain	203.00	SW(cyclical) (Hz)	24038.46	Solvent	CHLOROFORM-d
Sweep Width (Hz)	24037.73	Temperature (degree C)	24.460	Pulse Sequence	dept135
				Spectrum Offset (Hz)	10056.0332

mmmd_18_DEPT-135_010000fid.esp

Mass Spectrum SmartFormula Report

Analysis Info

Analysis Name D:\Data\PKH\AAO 917_RA6_01_12709.d
Method standard pos low.m
Sample Name AAO 917
Comment

Acquisition Date 12/3/2012 4:43:54 PM

Operator BDAL@DE
Instrument / Ser# micrOTOF-Q II 10205

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.3 Bar
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Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min
Scan End	1500 m/z	Set Collision Cell RF	300.0 Vpp	Set Divert Valve	Source

The cytotoxic activity of the isolated compounds.

Samples	HepG-2	PC-3	A549	MCF-7
	IC ₅₀ μ g/mL (%*)			
Aegeline	- (43.2)	- (10.4)	- (15.3)	73.7 (72.5)
<i>p</i> -hydroxybenzoic acid	- (32.7)	- (0)	- (32.2)	- (50.7)
Aegeline-B	- (36.1)	- (29.1)	- (36.5)	63.5 (78.5)
Total alkaloid fraction	52.9 (90.4)	- (23.9)	- (30.5)	59.5 (83.5)
Aegeline-A	71.4 (72.9)	72.5 (72.3)	- (29.6)	56.5 (81.2)
Adriamycin	21.6	23.8	28.3	26.1

LC₅₀: Lethal concentration of the sample which causes the death of 50 % of cells in 48 hrs.

* Inhibition percent (%) was recorded at 100 ppm.