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; ρ-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 $^1$H-$^1$H COSY correlations are indicated by bold bonds (▬) for compounds (1 & 2).

S1: $^1$H-NMR spectrum of compound (1)
S2: $^{13}$C-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: $^1$H-NMR spectrum of compound (2)
S9: $^{13}$C-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
| Parameter                  | Value       |
|---------------------------|-------------|
| Acquisition Time (sec)    | 1.9923      |
| Comment                   | 5 mm PABBO BB-1H/D Z-GRD Z108618/0050 |
| Date                      | 03 Oct 2012 08:19:12 |
| Date Stamp                | 03 Oct 2012 08:19:12 |
| File Name                 | D:\R- Projects\0- Denmark\Analysis of Samples\Full_NMR_Data_Denmark_2012\mmd_17\mmd_17_1H\mmd_17_1H_01000081fdd |
| Frequency (MHz)            | 400.12      |
| Nucleus                   | 1H          |
| Number of Transients      | 128         |
| Origin                    | spect       |
| Original Points Count     | 16384       |
| Owner                     | mmr         |
| Points Count              | 16384       |
| Pulse Sequence            | zg30        |
| Receiver Gain             | 203.00      |
| SW(cyclohexane) (Hz)       | 8223.68     |
| Solvent                   | MeOD        |
| Spectrum Offset (Hz)       | 1992.2266   |
| Sweep Width (Hz)           | 8223.18     |
| Temperature (degree C)     | 23.860      |
|                          |             |

Figure 16: 1H NMR spectrum of compound A.
| Acquisition Time (sec) | 13631 | Comment | 5 mm PABBO BB-1H/D Z-GRD Z108618/0050 | Date | 25 Oct 2012 00:38:24 |
|------------------------|-------|---------|--------------------------------------|-----|----------------------|
| Date Stamp             | 25 Oct 2012 00:38:24 |         |                                      |     |                      |
| File Name              | D:\Projects\0-Denmark\Analysis of Samples\Full_NMR_Data_Denmark_2012\mmd_17\mmd_17_13C\mmd_17_13C_010000fid |         |                                      |     |                      |
| Frequency (MHz)        | 100.62 | Nucleus | 13C | Number of Transients | 4086 | Origin | spect |
| Points Count           | 32768 | Owner   | nmr | Points Count | 32768 | Pulse Sequence | zgog30 |
| Receiver Gain          | 203.00 | SW(cyclic) (Hz) | 24038.46 | Solvent | MeOD | Spectrum Offset (Hz) | 10174.9248 |
| Sweep Width (Hz)       | 24037.73 | Temperature (degree C) | 25.060 |         |         |                     |
| Parameter                  | Value       |
|---------------------------|-------------|
| Acquisition Time (sec)    | 13631       |
| Comment                   | 5 mm PABBO BB-1H/D Z-GRD Z108618/0050 |
| Date                      | 30 Oct 2012 01:18:56 |
| File Name                 | Full_NMR_Data_Denmark_2012\mmd_18\mmd_18_13C\mmd_18_13C_010000fid |
| Frequency (MHz)           | 100.62      |
| Nucleus                   | 13C         |
| Number of Transients      | 8192        |
| Original Points Count     | 32768       |
| Owner                     | nmr         |
| Points Count              | 32768       |
| Pulse Sequence            | zgpg30      |
| Receiver Gain             | 203.00      |
| SW(cyclical) (Hz)         | 24038.46    |
| Solvent                   | CHLOROFORM-d |
| Spectrum Offset (Hz)      | 10056.7568  |
| Temperature (degree C)    | 24.960      |

**Diagram:**

![NMR Spectrum Diagram](image_url)

**Notes:**

mmd_18_13C_010000fid.esp
The cytotoxic activity of the isolated compounds.

| Samples               | HepG-2  | PC-3    | A549    | MCF-7  |
|-----------------------|---------|---------|---------|--------|
| Aegeline              | - (43.2)| - (10.4)| - (15.3)| 73.7 (72.5) |
| ρ-hydroxybenzoic acid | - (32.7)| - (0)   | - (32.2)| - (50.7) |
| Aegelbine-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) |
| Aegelbine-A           | **71.4 (72.9)** | **72.5 (72.3)** | - (29.6) | **56.5 (81.2)** |
| Adriamycin            | **21.6** | **23.8** | **28.3** | **26.1** |

IC$_{50}$: Lethal concentration of the sample which causes the death of 50% of cells in 48 hrs.

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