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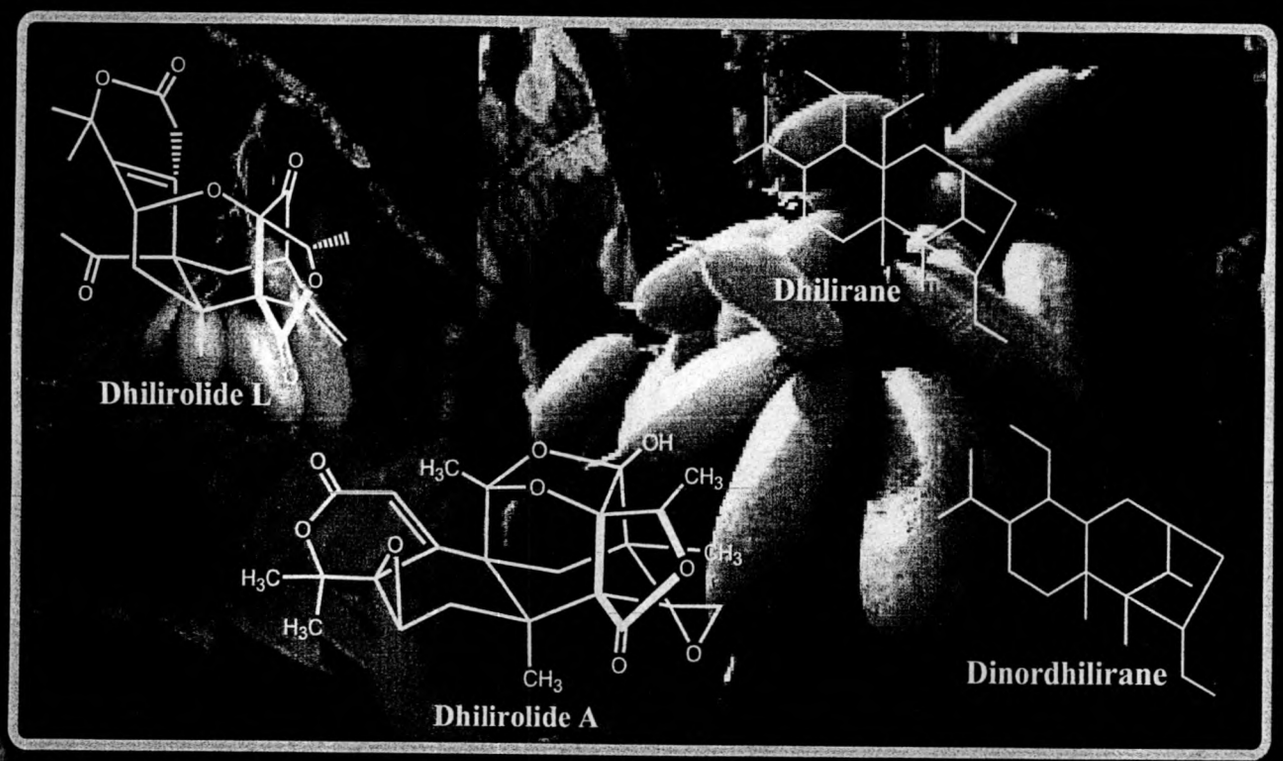

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Megastigmanes from Leaves of *Artocarpus heterophyllus* Lam.

K S S P Fernando[1], A M Abeysekera[1*], M I Choudhary[2], A K E Goonathilake[3], C Padumadasa[1], V M Thadani[4], A Adhikari[2], M Rehman[2], U G Chandrika[5]

[1.] *Department of Chemistry, Faculty of Applied Sciences, University of Sri Jayewardenepura, Nugegoda.*

[2.] *HEJ research Institute of Chemistry, International Center for Chemical and Biological Sciences, University of Karachi, Pakistan.*

[3.] *Department of Pharmacology, Faculty of Medical Sciences, University of Sri Jayewardenepura, Nugegoda.*

[4.] *Sri Lanka Institute of Nano Technology (pvt) Ltd. Nanotechnology & Science Park, Homagama.*

[5.] *Department of Biochemistry, Faculty of Medical Sciences, University of Sri Jayewardenepura, Nugegoda.*

[*]*Email: ajitabey@gmail.com*

Introduction

Artocarpus heterophyllus which belongs to the family Moraceae is a common tree in Sri Lanka. Medicinal properties of *A. heterophyllus* are well documented. In Sri Lankan traditional medicine the water extract of *A. heterophyllus* senescent leaves is used to reduce blood sugar levels. *Artocarpus heterophyllus* is a rich source of secondary metabolites such as flavonoids, stilbenes, triterpenes, chalcones, xanthonse and sterols. Most of the compounds that have been reported to date have been isolated from the root, wood and twigs. The chemistry of the leaves of *A. heterophyllus* has not been fully explored. Here we report two megastimane derivatives isolated from the senescent leaves of *A. heterophyllus*.

Materials and methods

Extraction

Water extract obtained from refluxing crushed *A. heterophyllus* senescent leaves (orange coloured) collected from Colombo district was concentrated under *vacuum*. Excess ethanol was added to precipitate the high molecular weight polysaccharides. After filtration the filtrate was concentrated under *vacuum*, extracted with ethyl acetate and solvent was removed under *vacuum* to produce a sticky solid (EA/W).

Fractionation

The sticky solid (EA/W) was chromatographed on Sephadex LH-20 eluting with five different solvent systems. Fraction 1 was eluted with dichloromethane/

hexane 4:1 and fractions 2, 3, 4 and 5 were eluted with dichloromethane/acetone 3:2, dichloromethane/ acetone 1:4, dichloromethane/methanol 1:1 and methanol, respectively. All fractions were collected separately and were subjected for *in vivo* hypoglyceamic activity studies, which revealed fractions 3 and 4 to be the most active. These two fractions had similar thin layer chromatographic profiles and were combined for compound isolation. Combined fraction was chromatographed on MCI gel column chromatography to produce 17 fractions (M1 – M17). Fraction M3 was chromatographed on silica using a gradient elution starting with 100% dichloromethane and gradually increasing the methanol concentration to 100%. A total of 130 fractions were collected. These fractions were combined based on their thin layer chromatographic profiles to produce 11 fractions (M3S1 – M3S11). M3S2 fraction was subjected to normal phase recycling preparative HPLC (ethyl acetate: hexane, 70: 30, 4 mL/min) to produce compound **(1)** in the pure form as a white solid. This was characterized by ^{1}H NMR, ^{13}C NMR, IR and UV-visible spectroscopy, FAB and HR-FAB mass spectrometry in positive ion mode.

^{1}H NMR (CD_3OD) 500 MHz δ: 0.81 (3H, H-12A, 12B, 12C), 0.85 (3H, H-13A, 13B, 13C), 1.08 (3H, H-11A, 11B, 11C), 1.47 (1H, H-2B), 1.71 (1H, H-2A), 2.10 (1H, H-5), 2.12 (1H, H-6), 2.26 (3H, H-10A, 10B, 10C), 3.57 (1H, H-4), 3.84 (1H, H-3), 6.06 (1H, H-8), 6.74 (1H, H-7).

^{13}C NMR (CD_3OD) 125 MHz δ: 17.4 (C-13), 24.0(C-11), 26.8 (C-10), 31.3 (C-5), 32.5(C-12, CH_3), 34.6 (C-1), 41.8 (C-2), 52.0 (C-6), 72.1 (C-3), 74.7 (C-4), 134.1 (C-8), 152.3 (C-7), 200.9 (C-9).

M3S5 fraction was subjected to normal phase recycling preparative HPLC (ethyl acetate: hexane, 70: 30, 4 mL/ min) and the fraction corresponding to the highest intense peak was collected. This was subjected to preparative thin layer chromatography with ethyl acetate: hexane (7: 3) as the solvent system. The band with R_f 0.4 was scrapped and stirred in methanol overnight and filtered. Filtrate was concentrated under *vacuum* at 45 °C. This was then purified by size exclusion recycling preparative HPLC (methanol, 4 mL/min) to produce compound **(2)** in the pure form as a white solid. This was then characterized by ^{1}H NMR, ^{13}C NMR, IR and UV-visible spectroscopy, FAB and HR-FAB mass spectrometry in positive ion mode.

^{1}H NMR (CD_3OD) 600 MHz: δ 6.13 (1H, m, H-4) , 5.64 (1H, H-7), 5.60 (1H, H-8), 4.25 (1H, H-9), 4.17 (1H, H-13A), 4.12 (1H, H-13B), 2.64 (1H, H-6), 2.49 (1H, H-2B), 2.10 (1H, 2A), 1.23 (3H, H- 10A, 10B, 10C), 1.02 (3H, H- 11A, 11B, 11C), 0.98 (3H, H-12A, 12B,12C)

^{13}C NMR (CD_3OD) 175 MHz δ 23.7 (C-10, CH_3), 27.3 (C-13, CH_3), 27.8(C-12, CH_3), 37.2 (C-1, C), 49.2 (C-2, CH_2), 52.1 (C-6, CH), 64.1 (C-11, CH_3), 68.8 (C-9, CH), 122.3 (C-4, CH), 127.4 (C-8, CH), 140.1 (C-7, CH), 168.3 (C-5, C), 202.0 (C-3, C)

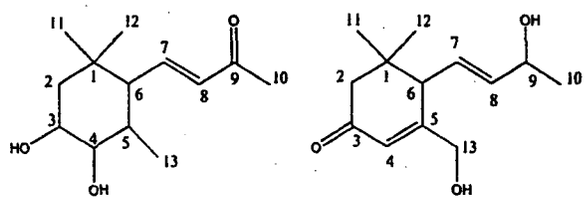
Results and Discussion

The EA/W fraction was subjected to repeated column chromatography over Sephadex LH-20, MCI gel, preparative TLC and preparative HPLC to yield compounds **(1)** and **(2)** in the pure form as white solids.

The molecular formula of compound **(1)** was determined as $C_{13}H_{23}O_3$ by HR-FAB mass spectrometry with the pseudo molecular ion $[M+H]^+$ peak observed at *m/z* 227.1640 (calculated for $C_{13}H_{23}O_3$, 226.1569). The ^{13}C NMR spectrum revealed 13 carbon signals in accordance with the molecular formula. These included the signals of four CH_3 carbons (C-10, C-11, C-12 and C-13), six CH carbons (C-3, C-4, C-5, C-6, C-7 and C-8) and one CH_2 carbon (C-2). The remaining carbon signals in the ^{13}C NMR spectrum are due to the conjugated keto carbonyl carbon (C- 9) and the quaternary carbon (C-1). Of the six CH carbons two are olefinic and are observed at 134.1 (C-8) and 152.3 (C-7). The other CH carbons are sp^3 carbons of which two are deshielded due to attachment of hydroxyl groups (C-4 and C-3). These assignments were confirmed by DEPT ^{13}C NMR spectrum of compound **(1)**. The ^{1}H NMR spectrum of compound **(1)** showed 12 hydrogen peaks. The four methyl signals were observed at $_{H}$0.81 (3H, s), 0.85 (3H, d, J = 6.5 Hz), 1.08 (3H, s) and 2.26 (3H, s). The spectrum showed two olefinic proton signals at $_{H}$6.06 (d, J = 15 Hz) and 6.74 (dd, J = 15Hz) and according to the coupling constants these are trans to each other. The spectrum also showed two oxymethine proton signals at $_{H}$ 3.57 (H-4) and 3.84 (H-3). The ^{1}H-^{1}H COSY spectrum of compound **(1)** showed all the important ^{1}H-^{1}H couplings. 1-D and 2-D NMR spectra confirmed compound **(1)** as 3,4-dihydroxy-7-ene-megastigman-9-one (Figure 1).

The molecular formula of compound **(2)** was determined as $C_{29}H_{26}O_8$ by HR-FAB mass spectrometry with the pseudo molecular ion $[M+H]^+$ peak observed at *m/z* 225.1450 (calculated for $C_{29}H_{27}O_8$, 224.1412). The ^{13}C NMR spectrum of compound **(2)** revealed 13 carbon signals in accordance with the molecular formula. These included the signals of three CH_3 carbons (C-10, C-11 and C-12), five CH carbons (C-4,

C-6, C-7, C-8 and C-9) and two CH_2 carbons (C-2 and C-13). The remaining two carbon signals in the ^{13}C NMR spectrum were assigned to the conjugated keto carbonyl carbon (C-3) and the quaternary carbon (C-1). Of the five CH carbons the olefinic carbons appeared at 122.3 (C-4), 127.4 (C-8), 140.1 (C-7) and 168.3 (C-5). The remaining CH carbon is a sp^3 carbon and is deshielded due to the attachment of the hydroxyl group. These assignments were confirmed by DEPT ^{13}C NMR spectrum of compound (**2**). The ^1H NMR spectrum of compound (**2**) showed 11 hydrogen peaks. The three methyl signals were observed at $_H$0.98 (H-12), 1.02 (H-11) and 1.23 (H-10). The spectrum showed three olefinic proton signals at $_H$6.13 (H-4), 5.64 (H-7) and 5.60 (H-8) and according to the coupling constants both C=C are in trans configuration. The ^1H-^1H COSY spectrum of compound (**2**) showed all the important ^1H-^1H couplings. 1-D and 2-D NMR spectra confirmed compound (**2**) as 9,13-dihydroxy-4,7-diene-megastigman-3-one (Figure 1).



Compound (1) Compound (2)

Figure 1. Structures of the megastignmane derivatives

Conclusion

Two megastigmane derivatives have been successfully isolated from ethyl acetate fraction of water extract of senescent leaves of *A. heterophyllus* upon extensive chromatography. They have been characterized by 1-D and 2-D NMR, IR and UV spectroscopy and HR-FAB mass spectrometry. The compounds have been previously reported, however, they have not been reported from *A. heterophyllus* species.

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Call for Nominations for Institute of Chemistry Gold Medal 2017 by 31st March *(Under Revised Rules)*

This Gold Medal was the very first of such awards to be donated to the Institute and was made possible through a generous donation made by **Mascons Ltd** in memory of their founder, **Mr A Subramanium** in 1978/79. It recognised contributions made to National Development through research and development involving Chemical Sciences. The Gold Medal Fund was supplemented recently through a further contribution from Mascons Ltd. This criteria governing the award were changed in 2011 since there were no applicants since 2007 in order to enable the award to be made to a mid-career Chemist in recognition of honorary services to the Institute.

Nominations are now being invited for the 2017 Award from amongst **Corporate Members** of the Institute who have fulfilled the following minimum criteria;

- Nominees should be not more than 55 years of age and should have been Corporate members of the Institute for at least 10 years on 1st of June 2017
- Nominees should have made significant contributions towards the activities of the Institute through yeoman services in an honorary capacity during the period of membership. These activities could include holding office, membership in committees, coordination of events such as workshops, social events etc.

Nominations could be made by any **corporate member** of the Institute and should include the consent of the nominee and details of the contributions made by the nominee in accordance with the above guidelines. The Award will be presented at the 46th Annual Sessions. **Nominations should be forwarded to reach the Hony. Secretary, Institute of Chemistry Ceylon not later than 31st March 2017.**