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RESEARCH NOTE

A new cytotoxic flabelliferin from palmyrah (*Borassus flabellifer* L.) flour

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INTRODUCTION

Palmyrah (*Borassus flabellifer* L.) flour (*Odiyal*) is known to have a number of toxins. These toxins possess mutagenic[1], clastogenic[2], immunosuppressive[3], dengue mosquito larvicidal[4] and neurotoxic[5] effects. *Odiyal* contains a number of saponins including a hyper-haemolytic factor[6], a variety of steroidal saponins (flabelliferins) and an antimicrobial flabelliferin F_B[7]. Many other flabelliferins are found in palmyrah fruit pulp[8] and palmyrah inflorescence[9]. The dominant aglycone in *odiyal* flour and palmyrah inflorescence is spirosterol[8,9].

During medium pressure liquid chromatography (MPLC) separation for isolating the dengue mosquito larvicide, a white amorphous solid was obtained. The objectives of this study were purification, identification and determination of bioactivity of this compound.

The ^{1}H and ^{13}C nuclear magnetic resonance (NMR) and two dimensional NMR experiments were conducted using Avance AV600 and Avance AV500 NMR spectrophotometers in diluted dimethyl sulfoxide (DMSO). The tetramethylsilane (TMS) signal was used as the internal standard. Fast atom bombardment mass spectra were obtained using a Varian MAT 312 mass spectrophotometer. The FMI Lab Pump QD with pre-absorbed silica packed MPLC column system and Chromatotron (Harrison Research, Australia) were used in chromatographic separations.

Palmyrah dried seed shoots were purchased from the Palmyrah Development Board. These seed shoots have been collected from home gardens in the Kalpitiya area during the year 2004. They were stored in a freezer (< 0°C) until use.

The cytotoxic compound 1 was isolated from palmyrah flour methanol: water (1:1) extracts, during the course of isolation of the mosquito larvicidal compound[4] using MPLC in petroleum ether: methylene chloride: ethyl acetate: methanol gradient at a flow rate of 18 mL/min against gravity. Further purification of compound 1 was conducted using chromatotron with methanol: water (1: 1) isocratic system. Compound 1 was re-crystallized using water, MeOH and EtOAc solvent mixtures and dissolved in DMSO at a concentration of 100 µg/mL. The resulting solution was introduced into micro-wells containing melanoma cancer cells obtained from the Institute of Cancer Research (ICR), USA. Doxorubicin 20 µg/mL solution was used as a positive control and the growth was measured microscopically after 24 h according to a well established screening method[10].

Compound 1 obtained as white coloured crystals (~ 10 mg, 0.001%) had a retention factor (Rf) value of 0.49 on thin layer chromatography (TLC) in butanol: ethanol: ammonia; (7: 3: 4) solvent system. The compound 1 was shown to have the molecular formula $C_{51}H_{82}O_{20}$ by high resolution fast atom bombardment mass spectrometry (HRFABMS) (negative mode) at m/z = (calculated: 1014.5399) found 1013.5318 for $[M - H]^-$. This shows the possibility of the presence of three-ramnosyl and a glucosyl residues, leaving room for

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Table 1: ^{1}H and ^{13}C NMR spectral data of compound 1

Carbon No	C-NMR δC	Multiplicity	^{1}H-NMR δH(J Hz)
1	37.6	CH2	2.14-2.38 (m)
2	29.0	CH2	1.58-1.59 (m)
3	76.2	CH	3.44 (m)
4	39.1	CH2	1.13-1.68 (m)
5	140.3	C	-
6	121.3	CH	5.38 (br s)
7	31.5	CH2	1.13-1.16 (m)
8	31.0	CH2	1.46 (m)
9	49.5	CH2	N/D
10	36.4	C	-
11	20.4	CH2	1.48 (m)
12	N/D	N/D	N/D
13	40.0	C	-
14	55.7	CH	1.06-1.07
15	31.5	CH2	1.87-1.94 (m)
16	80.3	CH	4.24-4.25 (br d)
17	61.8	CH	1.66 (m)
18	16.0	CH3	0.72 (s)
19	18.9	CH3	0.94 (s)
20	41.5	CH	1.76 (m)
21	14.6	CH3	0.89 (d, 6.9)
22	108.4	C	-
23	30.9	CH2	1.57 (m)
24	28.4	CH2	1.43 (m)
25	29.8	CH	N/D
26	65.9	CH2	3.18 (m)
27	17.3	CH3	0.73 (d 3.6)
1'	98.2	CH	4.38 (d 7.8)
2'	75.9	CH	N/D
3'	77.0	CH	3.19 (m)
4'	77.8	CH	N/D
5'	76.0	CH	N/D
6'	70.59	CH2	3.68 (br d)
1''	100.0	CH	4.66 (br s)
2''	70.4	CH	N/D
3''	70.7	CH	N/D
4''	75.3	CH	N/D
5''	67.9	CH	3.94-3.98 (m)
6''	N/D	N/D	N/D
1'''	100.3	CH	5.00 (br s)
2'''	71.3	CH	3.57 (m)
3'''	71.3	CH	3.55 (m)
4'''	80.2	CH	4.27-2.28 (m)
5'''	66.7	CH	3.94-3.98 (m)
6'''	18.9	CH3	0.944 (d)
1''''	101.1	CH	5.05 (br s)
2''''	-	-	-
3''''	70.6	CH	3.38 (m)
4''''	71.9	CH	3.17 (m)
5''''	68.9	CH	3.47 (m)
6''''	18.1	CH3	1.10 (d)

a steroidal aglycone of a MW of 414. Following peaks at m/z = 867 for $[M - Rha - H]^-$ and m/z = 721 for $[M - 2Rha - H]^-$ indicated the presence of two Rha groups.

The ^{1}H and ^{13}C NMR indicated following functions for aglycone moiety: four methyl [δ 0.72 (3H. s, 18-H_3), 0.73 (3H, d, *J*=3.6 Hz, 27-H_3), 0.89 (3H, d, *J*=3.6 Hz, 21-H_3), 0.94 (3H. s, 19-H_3)], a methylene δ 3.18 (2H, d-like, 26-H_2) and an olefin [δ 5.38, br peak, 6-H]. Anomeric protons were identified as β-glucopyranosyl [δ 4.37 (1H, d, *J*=7.8 Hz, 1'-H)] and three α- rhamnopyranosyl moieties [δ 4.66 (1H, br s, 1''-H), 5.00 (1H, br s, 1'''-H), 5.05 (1H, br s, 1''''-H)][9]. Glucose and rhamnose are commonly attached as the sugar moiety to palmyrah aglycones[8,9,11]. Generally, the 4th and 6th positions of the glucose 1, 4 linkage results in chemical shifts of 77-79 and 61.3-61.5 in pyridine[9]. However, 1, 6 linkage of the cytotoxic compound resulted in chemical shifts of 76.2 and 70.6 for the same linkage of the first glucose moiety. These results are comparable with previously reported data[12,13]. Spectroscopic data obtained for the cytotoxic compound confirmed the presence of two rhamnoses linked to the β glucose moiety with α 1, 2 and α 1, 6 linkages and another Rha'''' linked with α 1, 4 linkage to Rha'''. The following long-range correlations were observed between protons and carbons: 1'-H and 3-C; 2''-H and 1''-C; 1'''-H and 6'-C. Attachment of the sugars and spirostane type aglycone were confirmed by HMBC correlations except for the attachment of Rha'''' to Rha'''. Detail assignments of ^{1}H and ^{13}C-NMR data together with heteronuclear multiple quantum coherence (HMQC) and distortionless enhancement by polarization transfer (DEPT) were given in Table 1. Compounds with similar structures have been isolated from palmyrah inflorescence[9]. Data obtained during this study were comparable to that of above reported data. The structure of compound 1 was established as in Figure 1.

A -87% of growth was observed at a 100μg/mL concentration on the melanoma cell line, where Doxorubicin resulted a -37% at a concentration of 20 μg/mL. Thus, this experiment indicated cytotoxic effect on differentiating cancer cells. Logistic reasons prevented determining the anti cancer activity of the compound.

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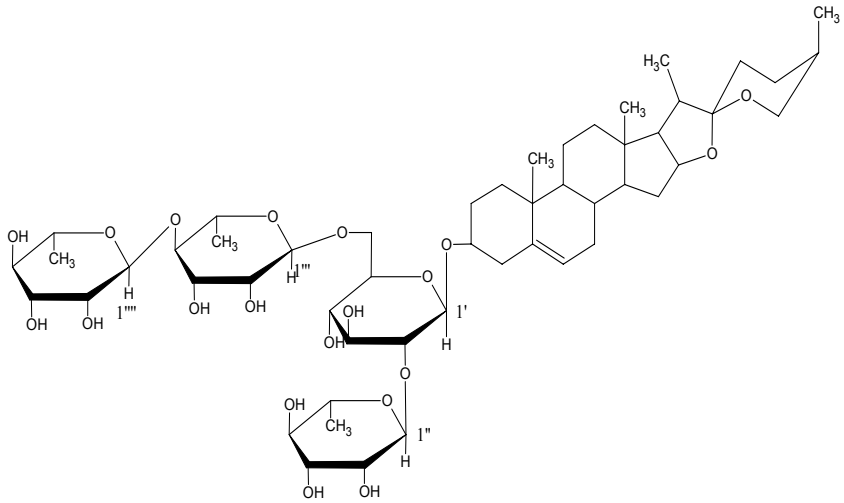

Figure 1: Structure of the cytotoxic compound 1

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