Isolation of Steroidal Saponin from *Dracaena umbratica*

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Abstract

Steroidal saponins are important class of components found in plants that play several crucial bioactivities based on previous studies. One known steroidal saponin was successfully isolated from *Dracaena umbratica*, a shrub of Dracaenaceae family. *Dracaena umbratica* also known as 'Senjuang' among local people. The isolation of a white amorphous steroidal saponin was conducted via normal phase vacuum liquid chromatography eluted with methanol. Its structure was established by spectroscopic analysis as 3-*O*-[α-L-rhamnopyranosyl (1→4)-α-L-rhamnopyranosyl (1→4)-β-D-glucopyranosyl]-25(R)-spirost-5-en-3β-ol.

Keywords: Dracaenaceae, *Dracaena umbratica*, Steroidal saponins

Introduction

The genus *Dracaena (Dracaenaceae)* includes more than 480 species distributed in tropical and subtropical dry climate region throughout the world (Mimaki et al., 1998 referred by Kougan et al., 2010). *Dracaena umbratica* is a shrub found in Malaysia and locally known as Senjuang. The decoction of the roots was reported as Malaysian folk medicine for rheumatism treatment (Burkill, 1969). The crude extract of the leaves and the roots of *Dracaena umbratica* showed high antioxidant activity when assayed by FTC assay (Zurina et al., 2010).

Previous studies showed that plants of genus *Dracaena* are synonym to steroidal saponins. Four new cyclosteroidal saponins were isolated from *Dracaena surculosa* by Yokosuka et al. (2002). The same group of researchers also found nine steroidal saponins from *Dracaena surculosa* two years before that. A total of ten steroidal saponins were successfully isolated from two species of *Dracaena* which are *Dracaena deisteliana* and *Dracaena arborea* by Kougan et al. (2010). Mimaki et al. (1998) had isolated thirteen steroidal saponins from the stems of *Dracaena concinna* while Tran et al. (2001) successfully isolated nine new steroidal saponins from the roots and rhizomes of *Dracaena angustifolia*. Xu et al (2010) worked on *Dracaena angustifolia* and succeeded in isolating six new steroidal saponins from the fresh stems of the species which they named as angudracanosides A-F. In another study, eighteen steroidal saponins were isolated from fresh stem of *Dracaena cochinchinensis* by Zheng et al. (2004) in which fourteen of them are claimed as new compounds. This paper describes the isolation of a steroidal saponin from the leaves of *Dracaena umbratic*a. One steroidal saponin has been isolated from the methanol extract of the leaves of this shrub.

Experimental section

Collection of plant sample

The plant materials (*Dracaena umbratica*) were collected from the area of Changlun in Kedah and also Ulu Pauh in Perlis. A voucher specimen was earlier prepared and identified by a botanist in UPM.

Preparation of plant extract

7.7 kg leaves of *Dracaena umbratica* was air dried for several days, ground to fine powder and macerated successively with solvents of increasing polarity (hexane, dichloromethane, methanol) for two days for each solvent. Methanol extract was then dried under

reduced pressure using rotatory evaporator (40^{o}C-47^{o}C) to give approximately 92 g of the plant extract.

Isolation of plant extract

150 g of normal phase silica gel P_{F254} (Merck) was packed in a column of diameter 10 cm matched to a receiver which is directly connected to a vacuum pump. Then, 20.0 g of the methanolic extract was impregnated with 40.0 g of normal phase silica gel, 230-400 mesh (Merck) and rotavap to dryness before it was subjected into the readily packed column. The impregnated sample was eluted with hexane, hexane : ethyl acetate (7:3), hexane : ethyl acetate (1:1), ethyl acetate, ethyl acetate : methanol (9:1), ethyl acetate : methanol (1:1). The column was finally eluted with methanol until clean. 100 ml of solvent system was applied every time of elution. Each fraction was collected in the separated conical flasks.

Results

42 mg of white amorphous solid was appeared in methanol fractions after vacuum liquid chromatography. It was then washed until clean. It is a non UV active compound with melting point = 287^{o}C and optical rotation, $[\alpha]^{25}$ = +0.462. IR : 3412.4 cm$^{-1,}$ 2929.7 cm^{-1}, 1043.4 cm^{-1} ; ESI-MS : [M+H] 867. Proton and carbon NMR results as listed in Table 1.

Discussions

IR spectrum exhibited the important signals of the glycosidic nature. A broad absorption band at 3412.4 cm^{-1} showed the presence of abundance of OH groups. The strong absorption at 1043.4cm^{-1} was referred to C-O stretch. Meanwhile the presence of C-H stretch was then revealed at frequency 2929.7 cm^{-1}.

The positive ion ESI-MS showed the quasimolecular ion peak at m/z 867 $[M+H]^{+}$, indicating a molecular weight of 866. The successive losses of two deoxyhexose and one hexose sugars were observed from the pattern of ion fragmentations, 867 $[M+H]^{+}$, 721 $[M+H+146]^{+}$, 575 $[M+H+(2x146)]^{+}$, 413 [M+H+(2x146)+162]. However, the OH at C-17 cannot be detected because it is unstable due to the attachment to a quartenary carbon (C-17). The molecular formula for this compound is $C_{45}H_{72}O_{17.}$

The complete structure of this compound was deduced based on data from spectroscopic techniques especially 1D and 2D NMR. Based on the NMR spectrum, it was confirmed to be a steroidal saponin with three sugar attachments. The aglycone skeleton is a type of spirostenol. The projected structure is supported by data from NMR spectrum and comparison with some other literatures (Agrawal et al. (1985), Mimaki et al. (1998), Yokosuka et al. (2000), Zheng et al. (2004)]. The strong characteristic of spirostenol steroidal saponin is due to the carbon at position 22 (C-22) whose value is 109.5 ppm. The presence of double bond was detected at 140.42 ppm (C-5) and 121.23 ppm (C-6) from carbon NMR spectrum. The proton at position 6 in NMR spectrum revealed the presence of double bond (5.40 ppm). Four methyl groups on the aglycone (0.84ppm;16.12ppm ; H-18;C-18), (1.06ppm;16.42ppm H-19;C-19), (0.91ppm;7.72ppm ; H-21;C-21), (0.81ppm;16.10ppm ; H-27;C-27) which are also the characteristic of spirostenol skeleton were successfully assigned from ^{1}H and ^{13}C NMR. The value of H-27 at the most upfield region (0.81 ppm) among other methyl protons provides an important information of the stereochemistry. In this case, it is claimed as R configuration.

Proton anomeric of hexose at 4.54 ppm in proton NMR showed a doublet with coupling constant value of 7.5 Hz revealed the presence of a β-D-glucopyranose sugar meanwhile the other two anomeric protons showed singlets at 4.91 ppm and 4.98 ppm belong to rhamnopyranose sugars. Two methyl groups in the sugar region showed coupling constant value of 6.5 Hz each, confirming the presence of rhamnopyranose sugars.

Based on the evidence from the spectroscopic analysis, it was confirmed that the structure of the steroidal saponin isolated from *Dracaena umbratica* is 3-*O*-[α-L-rhamnopyranosyl (1→4)-α-L-rhamnopyranosyl (1→4)-β-D-glucopyranosyl]-25(R)-spirost-5-en-3β-ol as illustrated in Figure 1. The Heteronuclear Multiple Quantum Correlation (HMQC) and Heteronuclear Multiple Bond Correlation (HMBC) correlation between proton and carbon can be referred to Table 1 and Figure 2.

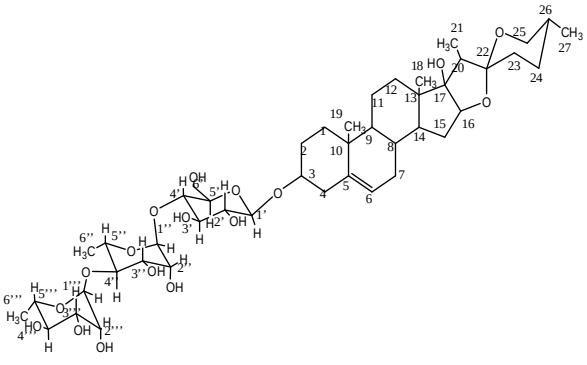

Figure 1. 3-*O*-[α-L-rhamnopyranosyl (1→4)-α-L-rhamnopyranosyl (1→4)-β-glucopyranosyl] -25(R)-spirost-5-en-3β-ol.

Table 1. ^{1}H and ^{13}C (HMQC) correlation (500 MHz, CD_3OD)

No. of carbon	^{13}C NMR	Multiplicity	^{1}H NMR	Coupling Constant (Hz)
1	37.9	CH_2	2.48, 2.33 m	
2	30.7	CH_2	2.01, 1.24 m	
3	77.6	CH	3.66, m	
4	37.2	CH_2	1.10, 1.91 m	
5	140.4	C	-	
6	121.2	CH	5.40, br d	4.0
7	31.8	CH_2	2.05, 1.58 m	
8	31.9	CH	2.01, m	
9	50.0	CH	0.95, m	
10	36.6	C	-	
11	20.1	CH_2	1.50, m (2H)	
12	31.4	CH_2	1.37, 1.38 m	
13	44.4	C	-	
14	52.5	CH	1.72, m	
15	31.1	CH_2	1.71, m (2H)	
16	89.1	CH	4.01, t	7.0
17	89.9	C	-	
18	16.1	CH_3	0.84, s	
19	18.4	CH_3	1.06, s	
20	44.1	CH	2.10, m	
21	7.7	CH_3	0.91, d	7.5
22	109.5	C	-	
23	29.9	CH	1.61, m	
24	28.0	CH_2	1.46, 1.61 m	
25	66.3	CH_2	3.34, 3.48 m	
26	29.3	CH_2	1.91, 1.30 m	
27	16.1	CH_3	0.80, d	6.5
1’	98.8	CH	4.54, d	7.5
2’	72.1	CH	3.43, m	
3’	78.0	CH	3.42, m	
4’	76.1	CH	3.27, m	
5’	68.7	CH	4.12, m	
6’	61.1	CH_2	3.65, 3.89 m	
1’’	101.3	CH	4.98, s	
2’’	70.7	CH	3.85, m	
3’’	70.9	CH	3.34, m	
4’’	86.9	CH	3.59, t like	9
5’’	69.1	CH	3.39, m	
6’’	16.4	CH_3	1.25, d (overlapped)	6.5
1’’’	102.4	CH	4.91, s	
2’’’	70.9	CH	3.34, m	
3’’’	71.0	CH	3.66, m	
4’’’	72.3	CH	3.42, m	
5’’’	69.4	CH	3.95, m	
6’’’	16.7	CH_3	1.28, d (overlapped)	6.5

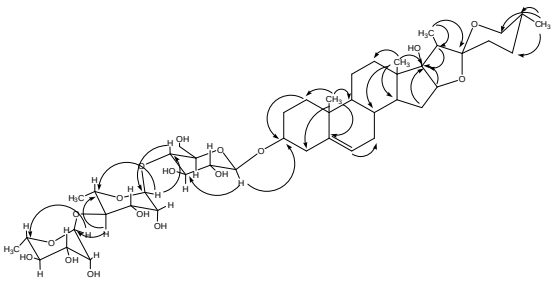

Figure 2. HMBC correlation (500 MHz, CD3OD)

Conclusions

The pure isolated compound was confirmed as a steroidal saponin in which is known as 3-*O*-[α-L-rhamnopyranosyl(1→4)-α-L- rhamnopyranosyl(1→4)-β-D-glucopyranosyl]-25(R)-spirost-5-en-3β-ol.

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