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Research Article

ISOLATION OF NEW GLYCOSIDES FROM PTERIDOPHYTE PLANT

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ABSTRACT

Two new glycosides have been isolated from ethyl acetate extract of the whole plant of *Actiniopteris radiata*. The ethyl acetate extract was subjected to column chromatography and isolated the active constituents. Two new glycosidic compounds were isolated and characterised by TLC, IR, UV spectral analysis, NMR and Mass spectra. Compound 1 is 2-(3, 4-O – Diglucos cinnamoyl) – 4 – hydroxyl furan and compound 2 is 1-Heptaloyl, 8-hexyl, 3-(O – diglucos), 10 – methyl, 9. 10 – dihydro naphthalene.

Keywords: Actiniopteris radiata, Polypodiaceae, Furan, glycoside

INTRODUCTION

Actiniopteris radiata is a desert fern belongs to the Polypodiaceae family and is distributed throughout India. A herbaceous miniature palm like fern up to 25 cm high with densely tufted stipe. Fronds fan like with numerous dichotomous segments which are rush like in texture, veins few, subparallel with distinct midrib, segments of fertile frond longer than those of the barren one, sori linear, elongate and submarginal. The plant is bitter, astringent, sweet, cooling, acrid, constipating, anthelmintic, haemostatic, antileprotic and febrifuge. It is useful in vitiated conditions of kapha and pitta, diarrhea, dysentery, helminthiasis, haemoptysis, haematemesis, leprosy, skin diseases, diabetes and fever. Plants are important source of new drugs and are also good lead compounds suitable for further modification during drug development[1]. Plant products and related drugs are used to treat 87% of all categorized diseases[2]. The secondary metabolites from natural products are showing more drug likeness and biologically friendliness than total synthetic molecules. In earlier days drug targets were exposed to crude extracts. But now a days the extracts were fractionated, active compounds isolated and characterized[3-5].

MATERIALS AND METHODS

Plant material

The whole plant of Actiniopteris radiata was collected from Nilgiri district, Tamil Nadu, India, in November 2007. The plant was identified by Dr. S. Rajan, Field Botanist, Survey of Medicinal Plants and Collection Unit, Emerald, Nilgiri. A voucher specimen has been deposited at Survey of Medicinal Plants and Collection Unit, Emerald, Nilgiri.

Instruments

Melting points were determined using a Lab India melting point apparatus. UV-Visible spectrums were recorded using a Shimadzu UV-1700. IR spectrums were recorded on a Shimadzu FTIR-8400s. ^{1}H (500 MHz) and ^{13}C (100 MHz) spectrums were recorded on a BRUKER AV-400. EIMS was recorded by GC-MS on a P-POS/TOP MICRO, HITACHI. ESIMS spectrums were recorded on a HCT-Ultra PTM discovery, BRUKER.

Extraction and isolation

The coarsely powdered plant material (500 g) was packed in soxhlet apparatus. The packed plant material extracted successively with petroleum ether, chloroform, ethyl acetate and ethanol for 18-20 hrs. These extracts were filtered and dried under vacuum[6]. The column chromatography was used as a purification technique for isolation of compounds from extracts. The ethyl acetate extract showed significant activity in the preliminary studies carried out and hence it was selected for further fractionation and isolation. Fractionations were carried out using silica gel column. The column was packed by wet packing method using petroleum ether as solvent. The extract dried under vacuum was found to be 16.0 g. It was packed in a column chromatography with a silica gel 60-120 mesh size as adsorbent (300.0 g). The mobile phase was allowed to flow through the column in the increasing order of polarity and fractions were collected. Thin layer chromatography was performed for all collected fractions and the fractions showing similar chromatograms were combined[7-9]. The eluents used were Petroleum ether 100, Petroleum ether: Chloroform (95:5, 90:10, 85:15, 80:20), Chloroform 100, Chloroform: Ethyl acetate (95:5, 90:10, 85:15, 80:20), Ethyl acetate 100, Ethyl acetate: methanol (95:5, 90:10, 85:15, 80:20). Sixteen fractions were collected. The purification was done for major fractions by re-column. The fraction 13 was evaporated to yield 380 mg of yellow residue. Fraction 13 (380 mg) was packed in column chromatography (silica gel 60-120 mesh size, 30 g). The solvents were allowed to flow in the order of increasing polarity. The eluents used were petroleum ether 10, petroleum ether: chloroform (9:1, 8:2, 7:3), chloroform 10, chloroform: ethyl acetate (9:1, 8:2, 7:3), ethyl acetate 10, ethyl acetate: methanol (9:1, 8:2, 7:3). Twelve fractions were collected. Fraction 11 yielded 45 mg of compound 1. The fraction 15 was evaporated to yield 120 mg of yellow residue. Fraction 15 (120 mg) was packed in column chromatography (silica gel 60-120 mesh size, 30 g). The solvents were allowed to flow in the order of increasing polarity. The eluents used were petroleum ether 10, petroleum ether: chloroform (9:1, 8:2, 7:3), chloroform 10, chloroform: ethyl acetate (9:1, 8:2, 7:3), ethyl acetate 10, ethyl acetate: methanol (9:1, 8:2, 7:3), methanol 10. Thirteen fractions were collected. Fraction 13 yielded 30 mg of compound 2.

2-(3, 4-O – Diglucos cinnamoyl) – 4 – hydroxyl furan (Compound 1)

A yellow solid; UV (CH_3OH) λ_{max}; 255 (2.65), 334 (1.41), 374 (1.44) nm; IR V_{max}: 3390, 3379, 3369, 3350, 1680, 1627, 1600, 1080 cm^{-1}; ^{1}H NMR (500 MHz, CD_3OD): δ 7.30 (*d*, 1H, *J*=16.0 Hz, H-3) and 6.70 (*d*, 1H, *J*=16.0 Hz, H-2), 5.70 (*d*, 1H, *J*=2.0 Hz, H-6) and 7.56 (*d*, 1H, *J*=2.0 Hz, H-8), 6.80 (*d*, 1H, *J*=7.0 Hz, H-5^1), 7.10 (*dd*, 1H, *J*=7.0 Hz, 2.0 Hz, H-6^1) and 6.25 (*d*, 1H, *J*=2.0 Hz, H-2^1); ^{13}C NMR (100 MHZ, CD_3OD): δ 171.48 (C=O), 166.60 (C-5), 150.28 (C-4^1), 161.56 (C-7), 147.23 (C-3^1) and 128.93 (C-1^1), 62.00 to 78.64 (two hexoses); (-) – ESIMS: m/z 570 [M$^+$], 408 [M-162]$^-$, 246 [M-2×162]$^-$ (calcd for $C_{25}H_{30}O_{15}$, 570.00).

1-Heptaloyl, 8-hexyl, 3-(O – diglucos), 10 – methyl, 9. 10 – dihydro naphthalene (Compound 2)

A yellow solid; UV (CH_3OH) λ_{max}: 256 (3.02), 318 (0.89), 334 (0.84) nm; IR V_{max}: 3369, 3329, 3315, 1734, 1670, 1035 cm^{-1}; ^{1}H NMR (500 MHz, CD_3OD): δ 0.9 (-CH_3), 1.29 (-CH_2-), 2.3 (3H), 3.0 (O-CH_2), 5.35 (H-8), 5.30 (H-7), 3.4 (H-3), 2.90 (H-2), 2.40 (H-1), 2.30 (H-2^1); ^{13}C NMR (100 MHz, CD_3OD): δ 130.86 and 129.02 (C=C), 185 (C=O),

11.00, 14.00 and 23.00 (3×CH_3), 26.00 and 43.45 (-CH_2-), 77.88 to 62.78 (two hexoses); (+) – ESIMS: m/z 680 [M^+], 702 [M+Na]$^+$ (calcd for $C_{36}H_{56}O_{12}$, 680.00).

RESULTS AND DISCUSSION

The ethyl acetate extract was chromatographed over silica gel using column chromatography to yield compound 1-2.

Compound 1, A yellow solid. The UV spectrum shows the absorption maximum at 255, 334 and 374 nm. IR spectrum exhibits characteristic bands at 3390, 3379, 3369, 3350 (-OH), 1680 (C=O), 1627, 1600 (C=C), 1080 (C-O) cm^{-1}. The Mass spectrum ESIMS in its negative mode spectrum showed a peak at m/z 570 for M^+. The peaks at m/z 408 for [M-162]$^-$ ion and at m/z 246 for [M-2×162]$^-$ ion confirms the presence of two glycosidic moieties. It shows the molecular formula of $C_{25}H_{30}O_{15}$ and molecular weight 570.00. Melting point 98^{o}C.

In the ^{1}H-NNR spectrum the signals at δ 6.70 (H-2) and 7.30 (H-3) are protons of –C=C-. They have a cross peak in the ^{1}H-^{1}H COSY spectrum. The signals at δ 5.70 (H-6) and 7.56 (H-8) are meta to each other and belongs to the furan ring. The signal at δ 7.11 (H-7) indicates the proton of hydroxyl group. The signal at δ 5.70 is in the upfield because it is between two carbon atoms C-5 and C-7 which contains hydroxyl group. Similarly the proton at H-8 appeared at δ 7.56. The signals at δ 6.80 (H-5^1), 7.10 (H-6^1) and at δ 6.25 (H-2^1) indicates protons of aromatic ring. They have cross peaks with each other in the ^{1}H-^{1}H COSY spectrum.

The ^{13}C-NMR spectrum has a signal at δ 171.48 for a carbonyl carbon, five signals at δ 166.60 (C-5), 150.28 (C-4^1), 116.92 (C-7), 147.23 (C-3^1) and 128.93 (C-1^1) are quaternary in nature. It indicates that two signals at 101.47 and 104.21 due to two anomeric carbon atoms C-1^{11} and C-1^{111} respectively. It was supported by the appearance of two anomeric hydrogen signals in ^{1}H-NMR at δ 5.30 (H-1^{11}) and 4.56 (H-1^{111}). The signals between δ 3.40 to 4.00 in the ^{1}H-NMR and between δ 62.00 to 78.64 in ^{13}C-NMR suggest the presence of two glycoside moieties in the compound. The glycosides are attached to a furochalcone nucleus. The above data suggests that it is a chalcone with two glycosidic moieties (two hexoses). Hence the structure of this compound is elucidated as 2-(3, 4-O – Diglucos cinnamoyl) – 4 – hydroxyl furan.

Table 1: NMR Spectral data of compound 1

Carbon	Signal (δ)	DEPT 135	Proton	Signal (δ)
2	117.59	up	H-2	6.70 *d*, 1H, *J*=16 Hz
3	136.86	up	H-3	7.30 *d*, 1H, *J*=16 Hz
4	171.48	--		
5	166.60	--		
6	92.55	up	H-6	5.70 *d*, 1H, *J*=2Hz
7	116.92	--	H-7	7.11 *d*, 1H, *J*=2Hz
8	161.56	up	H-8	7.56 *d*, 1H, *J*=2Hz
Aromatic carbon and Hydrogen				
1^1	128.93			
2^1	100.86	up	H-2^1	6.25 *d*, 1H, *J*=2Hz
3^1	147.23	-		
4^1	150.28			
5^1	117.40	up	H-5^1	6.80 *d*, 1H, *J*=7Hz
6^1	125.67	up	H-6^1	7.10 *d*, 1H, *J*=7Hz
Glycosidic carbon and Hydrogen				
Carbon	**Signal (δ)**		**Proton**	**Signal**
1^{11}	101.47		H-1^{11}	5.30
2^{11}	78.64		H-2^{11}	3.50
3^{11}	74.90		H-3^{11}	3.48
4^{11}	71.64		H-4^{11}	3.50
5^{11}	77.62		H-5^{11}	3.52
6^{11}	62.64		H-6^{11}	3.71, 3.90
1^{111}	104.21		H-1^{111}	4.56
2^{111}	78.52		H-2^{111}	3.50
3^{111}	74.89		H-3^{111}	3.52
4^{111}	70.95		H-4^{111}	3.40
5^{111}	77.61		H-5^{111}	3.52
6^{111}	62.21		H-6^{111}	3.70, 3.98

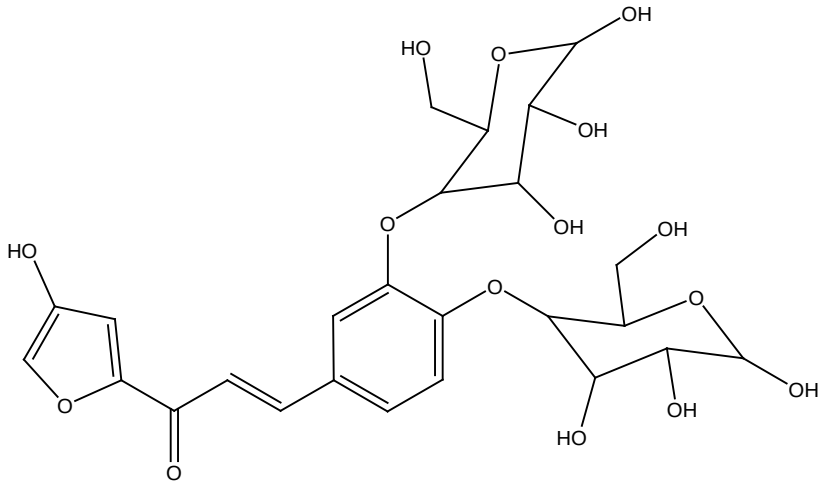
Compound 2, A yellow solid. The UV spectrum shows absorptions at 256, 318 and 334 nm. The IR spectra exhibits characteristic absorption at 3369, 3329, 3315 (-OH), 1734 (C=O), 1670 (C=C) and at 1035 (C-O) cm^{-1}. The positive mode ESIMS has peak at m/z 680 for M^+ ion and at m/z 702 for [M+Na]$^+$ ion. It shows the molecular formula of $C_{36}H_{56}O_{12}$ and molecular weight 680.00. Melting point 136^{o}C.

In ^{1}H-NMR, the multiplet at δ 0.9 for six protons indicates the presence of two methyl groups. They are terminal methyl groups of a long chain hydrocarbon. The two methyl groups suggests the presence of two long chain hydrocarbon groups. This was further supported by a broad singlet at δ 1.29. The complex multiplet signal at δ 2.3 (3H) indicates the presence of a methylene and a methyne group on either side of a carbonyl group. The presence of a two proton multiplet at δ 5.35 and 5.30 for proton adjacent to carbonyl group. Further it has two signals at δ 4.25 and 4.35 each for one proton indicating the presence of two anomeric protons indicates the presence of a disaccharide. This was completed by the signals between δ 3.65 and 4.10. The quateret signal at δ 3.00 for two protons indicates the presence of – O – CH_2 group supporting the above data.

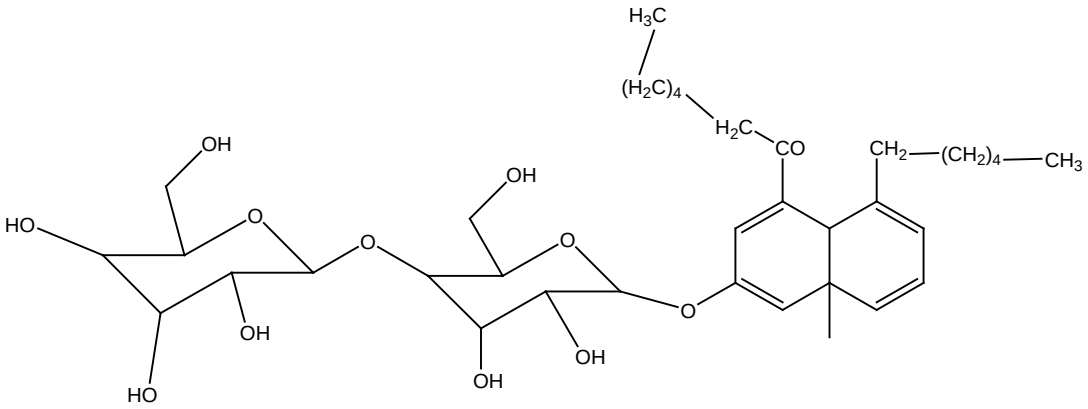
The ^{13}C-NMR spectra has the signals at δ 130.86 and 129.02 for the carbons adjacent to the carbonyl group, two anomeric carbon signals at δ 105.00 and 99.13, a carbonyl carbon at δ 185.00. The presence of signals at δ 11.00, 14.00 and 23.00 indicates the presence of three methyl groups. The group of signals between δ 26.00 and 43.45 indicates the presence of long chain hydrocarbons. The group of signals between δ 77.83 and 62.78 supports the presence of two hexose units. The spectral data of these two hexose units are given here. C-1^1 (105.00), C-1^{11} (99.13), C-2^1 (72.50), C-2^{11} (67.06), C-3^1 (77.83), C-3^{11} (74.92), C-4^1 (69.66), C-4^{11} (66.67), C-5^1 (73.42), C-5^{11} (71.64), C-6^1 (62.49) and C-6^{11} (62.78).

The protons and their respective carbon signals were established using HSQC spectra. The positions of the groups were fairly established based on the ^{1}H-^{1}H COSY spectra. A cross peak between

the signals at δ 5.35 (H-8) and 5.30 (H-7) suggesting that H-8 and H-7 are adjacent to each other. The signal at δ 5.30 (H-7) has a cross peak with a peak at δ 2.75 (H-6). Further there is no cross peaks for the proton H-10 suggesting that C-10 was connected to a quaternary carbon (C-5). The signal at δ 5.35 (H-8) has cross peak with a signal at δ 2.00 (H-9) which in turn has cross peak with a signal at δ 1.60. The cross peaks were observed between signals at δ 1.60, 1.29 and 1.29, 0.90. This strongly suggests that C-8 is connected to a long chain hydrocarbon. The cross peaks were observed between the following signals at δ 3.40 (H-3) and 2.90 (H-2); 2.90 (H-2) and 2.40 (H-1); 2.40 (H-1) and 1.29 (long chain methylene groups); 1.29 and 0.90 (CH_3 – group). The signal at δ 3.40 (H-3) suggests that the methene proton was under oxygen function and two glycoside units are attached to the oxygen at C-3. Hence the chemical name of the compound is 1-heptaloyl, 8-hexyl, 3-(O- diglucos), 10-methyl, 9, 10–dihydro naphthalene.



2-(3, 4-O–Diglucos cinnamoyl) – 4 – hydroxyl furan (Compound 1)



1-Heptaloyl, 8-hexyl, 3-(O–diglucos), 10 – methyl, 9. 10 – dihydro naphthalene (Compound 2)

Fig. 1: The structures of compounds 1-2

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