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Oligospirostanoside Saponin from *Asparagus filicinus* Fruits

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ABSTRACT

Methanolic extract of fresh, defatted fruits of Asparagus *filicinus* Buch-Ham. contains a complex mixture of steroidal saponins from which one new oligospirostanoside named as '**Filicinin-D**' has been isolated and characterised by various chemical as well as spectral studies as: 3-O-[{ β-D-galactopyranosyl (1 → 4)} { β-D-xylopyranosyl (1→6) }-β-D-glucopyranosyl (1→4) β-D-glucoopyranosyl] (25R)-spirost-5en-3β-ol.

Keywords: *Asparagus filicinus*, steroidal saponin, oligospirostanoside, Filicinin-D.

I. INTRODUCTION

The plant *Asparagus filicinus* Buch-Ham. commonly called *'Saunspaur'* and *'Sensarpal'* belongs to the family Liliaceae. It grows wild in Himachal Pradesh and Punjab (India). Plant is perennial tall, stem is hollow flexuous not much branched. Leaves are minute scales, spinscent, bearing in their axial needle like branchlets, called cladodes. Flower are white in colour and fruits-berries globes red-black upon ripen. The plant has been reported for its medicinal values[1] like vermifuge, taeniafuge, powerful diuretic, tonic astringent etc. Previously *Asparagus filicinus* plant has been reported for the presence of saponins[2-9] from roots, hence an attempt has been made to isolate and assign structure for saponin contents from fruits.

II RESULTS AND DISCUSSION

A new oligospirostanoside, Filicinin-D(**1**), obtained from concentrated methanolic extracts of fresh defatted fruits of *Asparagus filicinus*, after usual work up was separated by

column chromatography and recrystallised from large volumes of MeOH. The IR spectrum of this showed a well-defined spiroketal absorption bands[10-13]. Filicinin-D (**1**) showed positive results to Liebermann–Burchard [14-15] and negative to Ehrlich Reagent test [10, 16] indicating its spirostanolic nature.

Acid hydrolysis[17-19] of **1** furnished an aglycone- Diosgenin (mp, mmp, Co-TLC, EIMS, IR, its acetate) and the neutralised aqueous hydrolysate contained D-glucose , D-galactose and D-xylose (R_f and Co-PC). Enzymatic hydrolysis[11, 20] of the **1** with β-glucosidase liberated no β-D-glucose indicating that β-D-glucose is not the terminal sugar of the glycone moiety which is attached to aglycone-Diosgenin at C-3.

To find out the sequence of the sugars in glycone moiety, **1** was subjected to Kiliani hydrolysis[21]. The probe from the reaction mixture with the passage of time, on PC showed that D-galactose and D-xylose emerging out first must be the terminal sugars of sugar chain. Two D-glucoses molecules appearing out later are the inner sugars through which D-galactose and D-xylose are linked with aglycone- Diosgenin at C-3. The configurations of the sugars were deduced as 'β' by Klyne's Rule[22] as well as by ^{13}C-NMR data.[23-24]

1 was subjected to permethylation by modified Hakomori's method[11,25] and purified by CC to yield a permethylate, which on methanolysis followed by hydrolysis furnished four methylated sugars, identified by PC as: 2,3,6-tri-O-methyl-D-glucose ; 2,3-di -O-methyl-D-glucose ; 2,3,4-tri-O-methyl-D-xylose and 2,3,4,6-tetra-O-methyl-D-galactose . These results indicate that D-xylose and D-galactose are the terminal sugars linked with D-glucose at position 4 and 6 , which is linked with another molecule of D-glucose through (1→4) linkage, which in turn is attached with aglycone at C-3.

In order to determine the exact linkages of the sugars with each other, **1** was subjected to partial hydrolysis[26-28] to get four prosaponins PS_1 to PS_4. Acid hydrolysis of all these prosaponins furnished the same aglycone – Diosgenin but different sugars namely: D-glucose in PS_1 and PS_2; D-glucose, D-xylose in PS_3 and D-glucose, D–galactose in PS_4. Each prosaponin on permethylation followed by methanolysis and hydrolysis gave the following methylated sugars:

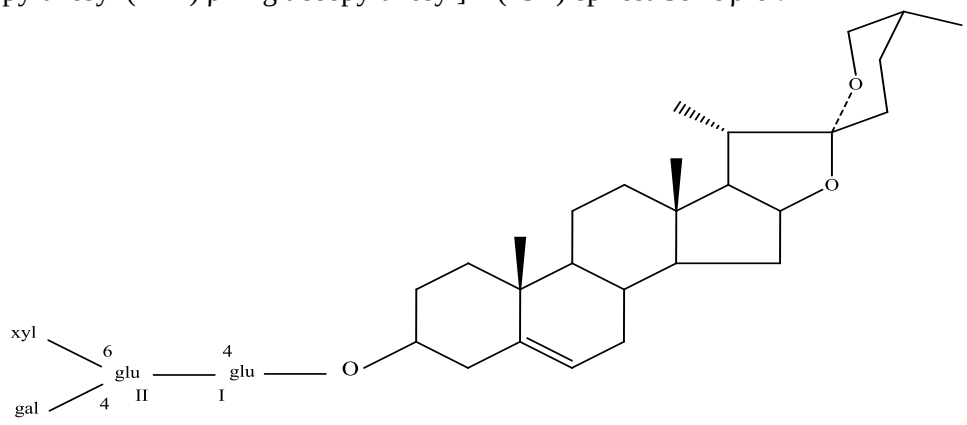
PS_1: 2,3,4,6-tetra-O-methyl-D-glucose; PS_2: 2,3,6-tri-O-methyl-D-glucose and 2,3,4,6-tetra-O-methyl-D-glucose; PS_3: 2,3,6-tri-O-methyl-D-glucose; 2,3,4-tri-O-methyl-D-glucose and 2,3,4-tri-O-methyl-D-xylose and PS_4: 2,3,6-tri-O-methyl-D-glucose (2 moles); 2,3,4,6-tetra-O-methyl-D-galactose.

Hence, PS_1 = Diosgenin + glucose (at C-3); PS_2 = PS_1 + glucose (1→4); PS_3 = PS_2 + xylose (1→6) and PS_4 = PS_2 + galactose (1→4). These results confirmed that D-galactose and D-xylose are the terminal sugars of sugar chain attached with D-glucose (II) at position 4 and 6 respectively, which in turn is linked with another molecules of D-glucose (I) through position 4 and finally with aglycone-Diosgenin at C-3.

FAB-MS of **1** showed molecular ion peaks at 1039 $[M+Li]^+$, indicating the presence of an aglycone of molecular weight 414 (Diosgenin), three molecules of hexoses (2 molecules of glucose and 1 molecule of galactose) and one molecule of pentose (xylose). ^{13}C-NMR data (**Table-I**) indicated that D-xylose and D-galactose are the terminal sugars of glycone moiety

attached to D-glucose (II) at C-4 and C-6 respectively, which further attached to D-glucose (I) at position 4.Thus spectroscopic data fully corroborates the earlier conclusions from chemical studies. All these results have thus proved the structure for **Filicinin-D (1**) beyond doubt as:

3-O-[{ β-D-galactopyranosyl (1 → 4)} { β-D-xylopyranosyl (1→6) }-β-D-glucopyranosyl (1→4) β-D-glucoopyranosyl] (25R)-spirost-5en-3β-ol.



glu = glucose, xyl = xylose, gal = galactose

Table-I ^{13}C-NMR Chemical Shifts of Sugar Moieties (D_2O)

Sugars	**Carbon Nos. Chemical shifts (ppm)**					
	1	**2**	**3**	**4**	**5**	**6**
Glucose I	103.2	72.4	78.6	70.2	78.6	61.3
Glucose II	103.3	72.1	78.5	69.4	81.7	61.0
Galactose	103.9	71.1	73.2	69.0	75.3	61.4
Xylose	104.2	73.4	76.4	69.8	66.0	--

III EXPERIMENTAL

The fruits of *Asparagus filicinus Buch-Ham*. were collected from village Hatwar, Dist. Bilaspur (HP), India. Extraction was carried out in open vessel at atmospheric pressure. CC was carried out over silica gel (60-120 mesh, BDH) with $CHCl_3$: MeOH solvent system in the order of increasing polarity. Homogeneity of the fractions was tested by TLC (silica gel-G, BDH with binder) and spots were visualised by 8-10% H_2SO_4 followed by heating. Melting points were determined in open capillaries in an electro thermal melting point apparatus. PC (descending) was carried out on Whatman Filter Paper No. 41 and spots were visualised by 'aniline hydrogen phthalate' reagent. IR, EIMS, FAB-MS and ^{13}C-NMR spectra were recorded

on Perkin Elmer, Jeol D-300, Jeol SX-102/DA-6000 (6KV, 10 mA, Acc. Volt. 10 KV) and Bruker WM-400 (400 MHz) respectively. The solvent systems used were:

A. $CHCl_3$: MeOH: H_2O (60: 35: 10)
B. C_6H_6: EtAc (8 : 2)
C. C_6H_6 : Pet. ether (1 : 1)
D. n-BuOH : AcOH : H_2O (4 : 1 : 5)
E. C_6H_6 : MeOH (9 :1)
F. n-BuOH : EtOH : H_2O (5 : 1 : 4)

Extraction and Isolation

The fresh fruits of *Asparagus filicinus* (1 kg) were defatted with pet. ether (4x6 h), ethyl acetate (3x6 h) and finally with MeOH (4x8 h). The methanolic extract was conc. under vac. to yield a brown mass. The brown mass was dissolved in minimum quantity of MeOH and then precipitated drop-wise-drop in large volumes of CH_3COCH_3 with constant shaking. The resulting residue was purified and separated by CC to get a new oligospirostanoside, Filicinin-D **(1)**

Filicinin-D (1)

1 was crystallised from MeOH (1.7 g)**;** mp 188-92 ^{o}C, $[\alpha]_D^{20}$ -68^{o} (MeOH), R_f 0.49 (Solvent- A) . It was positive to Liebermann-Burchard test and negative to Ehrlich reagent test. Its IR spectrum showed well defined spiroketal absorbance bands (900 > 918 cm^{-1}, 25R). FAB-MS showed molecular ion peak at 1039 $[M+Li]^+$ and ^{13}C-NMR data as in **Table-I**

Acidic Hydrolysis

Acidic hydrolysis of **1,** (100 mg) with 10% H_2SO_4 (50 ml) was carried out by refluxing (4 h) on a steam bath. After usual work up an aglycone was crystallised as colourless needles from MeOH; mp 203-206^{o}C, $[\alpha]_D^{20}$ -128 ^{o} ($CHCl_3$) [Diosgenin], R_f 0.56 (Solvent- B). $IR_{\upsilon\ max}^{KBr}$ cm^{-1} 3500-3400 (OH), 2846, 980, 918, 900, 860 (900 > 918, 25R). EIMS –m/z 414$[M]^+$, 396, 345, 300, 282, 271,253, 139 (base peak) and 115. Its acetate was prepared in cold in usual manner and crystallised as colourless needles from MeOH; mp 195-8 ^{o}C, $[\alpha]_D^{20}$ -119 ^{o} ($CHCl_3$) [Diosgenin acetate] , R_f 0.52 (Solvent –C) $IR_{\upsilon\ max}^{KBr}$ cm^{-1} OH(nil), 2845,980,918,898, 860.

The aqueous hydrolysate was neutralised with $BaCO_3$, filtered and concentrated under vacuum. PC studies (Solvent-D) revealed the presence of D-galactose (R_f 0.16), D-glucose (R_f 0.18) and D-xylose (R_f 0.28).

Enzymatic Hydrolysis

1 (50 mg) was taken up in distilled water (25 ml) and β-glucosidase (10 mg) was added along with toluene (3 drops) to cover the aqueous layer. The reaction mixture was kept at room temperature for 72 h. The PC (Solvent–D) did not show the presence of any sugar, whereas TLC (Solvent-A) showed one spot corresponding to **1** (R_f 0.49).

Kiliani Hydrolysis

1 (50 mg) was kept with Kiliani mixture 25 ml, (AcOH : H_2O : 35%HCl, 35 : 55 :10) at room temperature. PC (Solvent–D) after 24 h showed two spots corresponding to D-galactose (R_f 0.16) and D-xylose (R_f 0.28). PC after 48 h showed one more spot corresponding to that of D-glucose (R_f 0.18). PC after 72 h though showed the same number of spots but the intensity of D-glucose (R_f 0.18) spot was almost double. There was no change on PC after 96 h and even upon heating.

Permethylation

1 & 2 (250 mg) was permethylated by modified Hakomori's method (NaH, MeI, DMSO/N_2 atm.) to get permethyate (220 mg) which was purified by CC.

Methanolysis followed by hydrolysis

The above permethylate (200 mg) was refluxed with dry MeOH -1N HCl (50 ml) for 4 h on a steam bath, MeOH evaporated, H_2O (25 ml) was added and hydrolysed. After usual work up the aqueous neutralised hydrolysate on PC (Solvent-F) showed the presence of four methylated sugars as: 2,3,6 tri-O-methyl-D-glucose (R_G 0.83); 2,3 di -O-methyl-D-glucose (R_G 0.57); 2,3,4 tri-O-methyl-D-xylose (R_G 0.94) and 2,3,4,6 tetra-O-methyl-D-galactose (R_G 0.81).

Partial hydrolysis

1 (750 mg) was refluxed on a steam bath with 5% aq HCl- MeOH (50 ml, 1:1, 45 min.), neutralised by Ag_2CO_3 and filtered. The filtrate was dried under vac. and chromatographed to obtain an aglycone-Diosgenin (mp, mmp,Co-TLC) along with four prosaponins PS_1 to PS_4. Each prosaponin was acid hydrolysed and after usual work up showed only one aglycone- Diosgenin. The aqueous neutralised hydrolysates on PC (Solvent –D) showed sugars as: D-glucose (R_f 0.18) in PS_1 and PS_2 ; D-glucose (R_f 0.18), D-xylose (R_f 0.28) in PS_3 and D-glucose (R_f 0.18), D–galactose (R_f 0.16) in PS_4.

Each prosaponin on permethylation, methanolysis followed by hydrolysis and after usual work up of the neutral hydrolysate on PC (Solvent-E) showed different sugars : PS_1-2,3,4,6 tetra-O-methyl-D-glucose (R_G 1.00); PS_2-2,3,6 tri-O-methyl-D-glucose (R_G 0.83); 2,3,4,6 tetra-O-methyl-D-glucose (R_G 1.00); PS_3 -2,3,6 tri-O-methyl-D-glucose (R_G 0.83); 2,3,4 tri-O-methyl-D-glucose (R_G 0.85); 2,3,4 tri-O-methyl-D-xylose (R_G 0.94) and PS_4- 2,3,6 tri-O-methyl-D-glucose (2 moles, R_G 0.83); 2,3,4,6 tetra-O-methyl-D-galactose (R_G 0.81).

IV CONFLICT OF INTEREST

The author declares no conflict of interest.

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