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Oligofurostanosides-Furostanol Saponins from *Asparagus filicinus* Buch-Ham. Fruits

Naresh Kumar Thakur

Department of chemistry, Baba Balak Nath College Chakmoh, Dist. Hamirpur, (HP), India. Pin 176039.

(dr.naresh.thakur@gmail.com, 9418094120)

ABSTRACT

The methanol extracts of fresh fruits of *Asparagus filicinus Buch-Ham*. has been found to contain a complex mixture of steroidal saponins, out of which two new oligofurostanosides have been isolated and assigned the structure as: 3-O-[{ β-D-galactopyranosyl (1 → 4)}{ β-D-xylopyranosyl (1→6) }-β-D-glucopyranosyl (1→4)-β-D-glucopyranosyl]-26-O-β-D-glucopyranosyl-22α-methoxy- (25R)-furost-5-en, 3β, 26-diol (**Filicinoside-E**) and 3-O-[{ β-D-galactopyranosyl (1→4)}{ β-D-xylopyranosyl (1→6) }-β-D-glucopyranosyl (1→4)-β-D-glucopyranosyl] -26-O-β-D-glucopyranosyl- (25R)-furost-5-en,3β, 22α, 26-triol (**Filicinoside-F**) by chemical and spectral studies.

KEY WORD INDEX

Asparagus filicinus, steroidal saponins, oligofurostanosides

INTRODUCTION

Asparagus filicinus (Family-Liliaceae) commonly called *'Saunspaur', 'Sensarpal' and 'Sansarbuti'*, a wild growing plant of Himachal Pradesh and Punjab (India) has been reported for its medicinal values [1] as: vermifuge, taeniafuge, powerful diuretic, antipyretic, antitussive, expectorant, stomachic, nervous stimulant, tonic etc. It is a perennial herb, commonly seen growing in roadsides, pathways, forests, thickets in shady and moist places. It is also grown as an ornamental plant in gardens. Young shoot, green cladodes and roots of *Asparagus filicinus* are edible. So it is harvested from the wild habitat for local use as a food and medicine. *Asparagus filicinus is* an erect herb with a short rhizome and a bunch of fleshy swollen fasciculate roots. Its stem is hollow flexuous not much branched. Leaves are minute scales, bearing in their axial needle like branchlets, called 'cladodes'. Flowers are white in colour and fruits red-black -berries globes upon ripen. Previously *Asparagus filicinus* plant has been reported for the presence of saponins [2-9**]** mainly from roots, hence an attempt has been made to isolate and assign structure for saponin contents from fruits.

EXPERIMENTAL

The fruits of *Agave vera-cruz* Mill. were collected from village Hatwar, Dist. Bilaspur (HP), India. Extraction was carried out in an 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 and Ehrlich Reagent 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 : 50 : 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. n-BuOH : EtOH : H_2O (5 : 1 : 4) **F**.C_6H_6 : MeOH (9 :1)

Extraction and Isolation

The fresh fruits (1kg) of *Asparagus filicinus* were extracted with pet. ether (4x6 hrs.), ethyl acetate (2x7 hrs.) and finally with MeOH (4x8 hrs.). The methanol extract was conc. under vac. and extracted with n-BuOH. n-BuOH extract was dried under vac. and dissolved in minimum quantity of MeOH, then precipitated drop wise in large volumes of acetone with constant shaking. The resulting residue was purified and separated by CC to get two new oligofurostanosides, **Filicinoside-E(1) & F(2).**

Filicinoside-E(1) & F(2).

Inseparable mixture **1 & 2** (2.3 g) by CC, showed no spiroketal absorbance in the IR spectrum and gave all the characteristic tests for furostanosides .

Filicinoside-E(1)

1 & 2 mixture (100 mg) was refluxed with dry MeOH (50 ml) for 6 hrs. on a water bath to yield **1**. mp 170-6 ^{o}C, $[\alpha]_D^{20}$ -63^{o} (MeOH), R_f 0.66 (Solvent- A).

Filicinoside-F(2)

1 & 2 mixture (100 mg) was refluxed with aqueous acetone (50 ml, 1:1) for 8 hrs. on a water bath to yield **2**. mp 164-8 ^{o}C, $[\alpha]_D^{20}$ -64.5^{o} (Py), R_f 0.47 (Solvent- A).

Acidic Hydrolysis

Acidic hydrolysis of **1, 2** mixture (100 mg) with 10% H_2SO_4 (50 ml) was carried out by refluxing for 4 hrs. on a steam bath. The usual work up furnished an aglycone, 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 aq. hydrolysate was neutralised with $BaCO_3$, filtered and conc. under vac. 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 & 2 (50 mg) was taken up in distilled water (25 ml) and β-glucosidase (10 mg) was added to it along with toluene (3 drops) to cover the aq. layer. The reaction mixture was kept at room temperature for 72 hrs. The PC (Solvent–D) showed the presence of a D-glucose (R_f 0.18).

Kiliani Hydrolysis

1 & 2 (50 mg) was kept with Kiliani mixture 25 ml, (AcOH : H_2O : 35%HCl, 35 : 55 :10) at room temp. PC (Solvent –D) after 3 hrs. showed the presence of D-glucose(R_f 0.18) . PC after 24 hrs. showed two additional spots corresponding to D-galactose (R_f 0.16) and D-xylose (R_f 0.28). PC after 48 hrs. and 72 hrs. though showed the same number of spots but the intensity of D-glucose's spot was almost double and triple respectively . There was no change on PC after 96 hrs. 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, R_f 0.84 (Solvent –F)} 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 hrs. on a steam bath, MeOH evaporated, H_2O (25 ml) was added and hydrolysed. After usual work up the aq. neutralised hydrolysate on PC (Solvent-E) showed the presence of 2,3,4,6 tetra-O-methyl-D-glucose (R_G 1.00); 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 &2 (1 g) was refluxed on a steam bath with 5% aq HCl- MeOH (50 ml, 1:1, 45 min.), neutralised (Ag_2CO_3) and filtered. The filtrate was dried under vac. and chromatographed to obtain an aglycone-Diosgenin (mp, mmp,Co-TLC) along with five prosaponins PS_1 to PS_5. Each prosaponin was acid hydrolysed and usual work up showed only one aglycone- Diosgenin. The aq. neutralised hydrolysates on PC (Solvent –D) for sugars showed as: D-glucose (R_f 0.18) in PS_1 , PS_2 and PS_3; D-glucose (R_f 0.18), D-xylose (R_f 0.28) in PS_4 and D-glucose (R_f 0.18), D –galactose (R_f 0.16) in PS_5. Out of these prosaponins only PS_3 was positive to Ehrlich reagent test indicating its furostanolic nature.

Each prosaponin was subjected to permethylation and methanolysis followed by hydrolysis. After usual work up PC (Solvent-E) of the neutral hydrolysate showed different sugars viz. 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_3-2,3,4,6 tetra-O-methyl-D-glucose (2 moles , R_G 1.00); PS_4-2,3,6 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_5- 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).

RESULTS AND DISCUSSION

The concentrated methanolic extract of the fresh fruits of *Asparagus filicinus* yielded two new oligofurostanosides, **Filicinoside-E(1) & F(2)** which could not be separated by column chromatography. The IR spectrum of this inseparable mixture of **1** & **2** showed no characteristic spiroketal absorption bands [10-13] and gave positive results with Liebermann–Burchard [14-15] and Ehrlich Reagent test [10,16] indicating its furostanolic nature**.** The mixture **1** & **2** , on refluxing with dry methanol provided **Filicinoside-E(1),**while on refluxing with aqueous acetone yielded **Filicinoside- F(2)**. Both these compounds gave all the characteristic tests of oligofurostanosides [10-16].

Enzymatic hydrolysis [11,17] of the mixture **1** & **2** with β- glucosidase liberated β-D-glucose revealing D-glucose to be the terminal sugar and a prosaponin negative to Ehrlich reagent test .This revealed that β-D-glucose is liberated from C-26 of the oligofurostanoside resulting the closure of F-ring and formation of corresponding oligospirostanoside (prosaponin). Acid hydrolysis [18-20] of **1 & 2** afforded an aglycone- Diosgenin (mp, mmp,

Co-TLC, EIMS, IR, its acetate) and the aq. neutralised hydrolysate contained D-galactose, D-glucose and D-xylose (R_f and Co-PC).

In order to find out the sequence of the sugars, **1 & 2** was subjected to Kiliani hydrolysis [21]. Examination of the reaction mixture with the passage of time on PC showed that D-glucose appeared first must be the sugar attached C-26 since the resulted reaction mixture was negative to Ehrlich reagent test. D-xylose, D-galactose emerging out then, must be the terminal sugars of another sugar chain. Two glucose molecules emerging out later are the inner sugars through which D-xylose, D-galactose are linked to the aglycone – Diosgenin at C-3 . The configurations of the sugars were deduced as 'β' by Klyne's Rule [22] as well as from ^{13}C-NMR data [23-24].

1 & 2 was permethylated by modified Hakomori's method [11,25] to get a permethylate, which on methanolysis followed by hydrolysis furnished methylated sugars, identified by PC as 2,3,4,6 tetra-O-methyl-D-glucose ; 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 clearly established that D-xylose and D-galactose are the terminal sugars of sugar chain linked through two molecules of D-glucose attached with aglycone at C-3 . The rest D-glucose is the sugar moiety attached at C-26 of aglycone.

In order to establish the exact linkages of the sugars with each other, **1** & 2 was subject to partial hydrolysis [26-28] to get five prosaponins PS_1 to PS_5. Acid hydrolysis of these prosaponins furnished the same aglycone –Diosgenin but different sugars viz. D-glucose in PS_1, PS_2 and PS_3; D-glucose , D-xylose in PS_4 and D-glucose , D –galactose in PS_5. Out of these prosaponins only PS_3 was positive to Ehrlich reagent test indicating its furostanolic nature hence indicating D- glucose moieties at C-3 and C-26. 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; 2,3,4,6 tetra-O-methyl-D-glucose; PS_3-2,3,4,6 tetra-O-methyl-D-glucose (2 moles) ; PS_4-2,3,6 tri-O-methyl-D-glucose ; 2,3,4 tri-O-methyl-D-glucose ; 2,3,4 tri-O-methyl-D-xylose and PS_5-2,3,6 tri-O-methyl-D-glucose (2 moles); 2,3,4,6 tetra-O-methyl-D-galactose .

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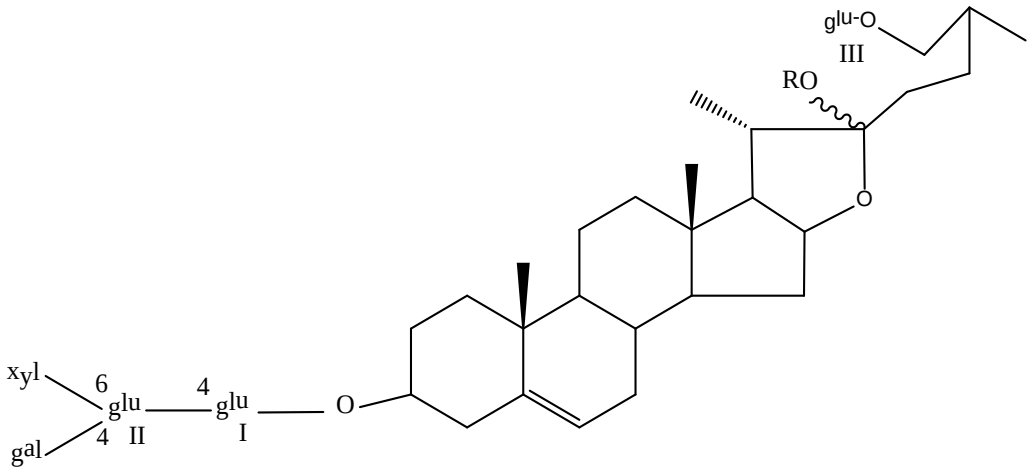
Hence, PS_1 = Diosgenin + glucose (at C-3); PS_2 = PS_1 + glucose (1→4); PS_3 = PS_1 + glucose (at C-26 and 22 α-OMe/-OH); PS_4 = PS_2 + xylose (1→6) and PS_5 = PS_2 + galactose

(1→4). These results confirmed a branching in the sugar chain at glucose (No. II) with D-xylose (1→6) and D-galactose (1→4) linkages. FAB-MS of **1 & 2** showed molecular ion peaks at 1233 $[M+Li]^+$, indicating the presence of an aglycone of molecular weight 414(Diosgenin), four molecules of hexoses (3-glucose and 1-galactose) and one molecule of pentose (xylose) along with open F-ring (22-OMe). ^{13}C-NMR data (**Table-I**) further confirmed these results as:

3-O-[{ β-D-galactopyranosyl (1 → 4)}{ β-D-xylopyranosyl (1→6) }-β-D-glucopyranosyl (1→4)-β- D-glucopyranosyl]-26-O-β-D-glucopyranosyl-22α-methoxy- (25R)-furost-5-en, 3β, 26-diol (Filicinoside-E) and 3-O-[{ β-D-galactopyranosyl (1→4)}{ β-D-xylopyranosyl (1→6) }-β-D-glucopyranosyl (1→4)-β-D-glucopyranosyl] -26-O-β-D-glucopyranosyl-(25R)-furost-5-en,3β, 22α, 26-triol (Filicinoside-F)



1. R= Me
2. R= H

Table-I

^{13}C-NMR CHEMICAL SHIFTS OF SUGAR MOIETIES IN D_20

Sugars	**Carbon Nos. Chemical shifts (ppm)**					
	1	**2**	**3**	**4**	**5**	**6**
Glucose I	103.1	72.2	78.6	70.2	78.6	61.2
Glucose II	103.4	72.1	78.6	69.5	81.8	61.0
Galactose	103.9	71.0	73.2	68.9	75.2	61.3
Xylose	104.2	73.4	76.5	69.8	66.0	-
Glucose III	103.3	73.5	75.2	70.2	75.3	61.4

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REFERENCES

[1] Kirtikar, K. R. and Basu, B. D. (1975). *Ind. Med. Plants*, Period.Expt. , Delhi, Vol. IV: 2466
[2] Sharma, S. C. and Thakur, N. K. (1994). *Phytochem*. 36:469
[3] Sharma, S. C. and Thakur, N. K. (1996). *Phytochem*. 41: 599
[4] Zhou, L –B, Chen, T –H, Bastow, K. F., Shibano, M. K., Lee, H. and Chen, D –F. (2007). *J.Nat.Pro*., 70(8) : 1263
[5] Li, Y –F., Hu, L –H., Lou, F –C., Hong, J –R., Li, J. and Shen, Q. (2005). *J.Asian Nat. Prod.Res.*,7(1) : 43
[6] Zhou, L –B. and Chen, D –F. (2008). *Steroids*, 73(1):83
[7] Wu, J –J., Cheng, K –W., Zuo, X –F., Wang, M –F., Li, P., Zhang, L –Y., Ye,W –C. (2010). *Steroids*, 75(10): 734
[8] Zhou, L., Cheng, Z. , Chen, D. (2012). *Aceta Pharma.Sinica B*, 2(3): 267
[9] Thakur,N.K. (2021). *J . Chem. & Cheml. Sci.* 11(2):19.
[10] Kiyosawa, S. and Hutoh, M. (1968). *Chem. Pharm. Bull*., 16: 1162
[11] Tschesche, R., Seidel, L., Sharma, S. C. and Wulff, G. (1972). *Chem. Ber*., 105: 3397
[12] Kawasaki, T., Komori, T., Miyahara, K., Nohara, T., Hosokawa, I., Ichiro, M. and Mihoshi, K.(1974). *Chem. Pharm. Bull.* , 22**:** 2164
[13] Dragalin, I. P. and Kintya, P. K. (1975). *Phytochem*., 14: 1817
[14] Lieberman, G. (1885). *Ber.Deut.Chem.Ges.*, 18: 1804
[15] Burchard, H. (1890). *Chem.Zentbl*., 1: 25
[16] Konishi, T., Kiyosawa, S. and Shoji, J. (1985). *Chem. Pharm. Bull*., 33:591
[17] Macek, K. (1963). *Paper Chrom*. Academic Press, New York: 289
[18] Gulbaran, E.(1964). *J. Am. Leather Chem. Ass.* , 59: 619
[19] Scoddast, R. W., Barrett, A. J. and Northcote, D. H.(1967). *J .Biochem*., 102: 194
[20] Tschesche, R., Ludke, G. and Wulff, G.(1967). *Tet. Lett*.: 2785
[21] Kiliani, H. (1930). *Ber*. , 63: 2866
[22] Klyne, W. (1950). *Biochem.J.* , 47: 41
[23] Agrawal, P. K., Jain, D. C., Gupta, R. K. and Thakur, R. S.(1985). *Phytochem*., 24: 2479
[24] Agarwal, P. K. (1992). *Phytochem.*,31(10): 3307
[25] Hakomori, S. (1964). *J. Biochem*., 55: 205
[26] Kuhn, R. and Low, I. (1963). *Liebig's Ann. Chem*., 669: 183
[27] Tschesche, R., Inchaurrondo, F. and Wulff, G. (1964). *Liebig's Ann. Chem*., 680: 107
[28] Hensens, O. D. and Lewis, K. G. (1965). Tet. *Lett*.: 4639