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SPIROSTANOL SAPONINS FROM *PARIS POLYPHYLLA*, STRUCTURES OF POLYPHYLLIN C, D, E AND F*

SHEO B. SINGH, RAGHUNATH S. THAKUR and HANS-R. SCHULTEN†

Central Institute of Medicinal and Aromatic Plants, Lucknow, 260010, India; †Institute of Physical Chemistry, University of Bonn, Bonn, West Germany

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Key Word Index—*Paris polyphylla*; Liliaceae; diosgenin; saponins; diosgenin-3-*O*-α-L-rhamnopyranosyl (1→3)-β-D-glucopyranoside; diosgenin-3-*O*-α-L-rhamnopyranosyl (1→3)[α-L-arabinofuranosyl(1→4)]β-D-glucopyranoside; diosgenin-3-*O*-α-L-rhamnopyranosyl(1→2)-α-L-rhamnopyranosyl(1→4)][α-L-rhamnopyranosyl(1→3)]-β-D-glucopyranoside; diosgenin-3-*O*-α-L-rhamnopyranosyl(1→4)[α-L-rhamnopyranosyl-(1→3)][β-D-glucopyranosyl(1→2)]α-L-rhamnopyranoside.

Abstract—The structures of four new saponins, polyphyllin C, D, E and F, isolated from the tubers of *Paris polyphylla* have been elucidated as diosgenin-3-*O*-α-L-rhamnopyranosyl(1→3)-β-D-glucopyranoside, diosgenin-3-*O*-α-L-rhamnopyranosyl(1→3)-[α-L-arabinofuranosyl(1→4)]-β-D-glucopyranoside, diosgenin-3-*O*-α-L-rhamnopyranosyl(1→2)-α-L-rhamnopyranosyl(1→4)[α-L-rhamnopyranosyl(1→3)]-β-D-glucopyranoside and diosgenin-3-*O*-α-L-rhamnopyranosyl(1→4)[α-L-rhamnopyranosyl(1→3)][β-D-glucopyranosyl(1→2)]-α-L-rhamnopyranoside, respectively, on the basis of chemical and spectral data.

INTRODUCTION

In a preliminary communication we reported the isolation of new saponins from *Paris polyphylla* [1]. The present paper deals with the structure elucidation of the compounds polyphyllin C, D, E and F.

RESULTS AND DISCUSSION

Polyphyllin C (**1a**), $C_{39}H_{62}O_{12}$, 745 (FDMS [16] $[M+Na]^+$), mp 185–190° (decomp.) and polyphyllin D (**2a**), $C_{44}H_{70}O_{16}$, 877 (FDMS $[M+Na]^+$), mp 227–230° (decomp.) were shown to be the spirostanol diglycoside and triglycoside of diosgenin respectively. Acid hydrolysis gave the compounds indicated in Table 1. Both compounds showed spiroketal absorptions ($920 > 900\ cm^{-1}$) in their IR spectra [2] and both gave a negative Ehrlich response [3, 4]. Partial hydrolysis of **2a** afforded polyphyllin C and diosgenin-3-*O*-β-D-glucopyranoside (**3a**). The latter compound was also obtained when polyphyllins C and D were subjected to Smith degradation [5, 6].

The inter-sugar linkage in polyphyllin C was established as (^{1}rha→^{3}glu) on the basis of the Kiliani hydrolysis of its hexamethylate (**1b**) and results of Smith degradation.

The configuration at the anomeric carbon was assigned as α to L-rhamnose and β to D-glucose on the basis of (a) ^{1}H NMR spectral data (Table 2) which showed coupling constants of 5.5 and 8 Hz for the axial anomeric proton in $+4_{C_1}$ conformation, and (b) Klynes' rule of molecular rotation (Table 3) [7]. Thus, polyphyllin C may be assigned the structure diosgenin-3-*O*-α-L-rhamnopyranosyl(1→3)β-D-glucopyranoside.

The mass spectra of the octamethyl ether (**2b**) and octa-acetate (**2c**) of polyphyllin D showed prominent peaks at *m/z* 189 (trimethyl rhamnose), 175 (trimethyl arabinose), 413 (diosgenin-H), 397 (diosgenin-OH), 273 (triacetyl rhamnose), 259 (triacetyl arabinose) which confirmed that both rhamnose and arabinose were attached as end sugars through a glucose residue.

The location of the arabinose moiety in polyphyllin D was determined by Kiliani hydrolysis of the octamethyl ether (**2b**) and periodate oxidation of the methylated sugars. As the latter reaction did not furnish a di-*O*-methyl-D-glucose, the glucose moiety in polyphyllin D must be substituted at C-3 and C-4 which suggested that the arabinose residue is attached to C-4. Arabinose was found to be present in the furanose form on the basis of the following evidence: (1) Kiliani hydrolysis afforded 2,3,5-tri-*O*-methyl-L-arabinose. (2) Partial hydrolysis did not yield any glycoside containing arabinose, indicating its easy hydrolytic fission [8]. The ^{1}H NMR spectral data (Table 2) and Klynes' rule of molecular rotation proved the anomeric configuration to be α, α, β with respect to L-rhamnose, L-arabinose and D-glucose, respectively. Thus the structure of polyphyllin D may be assigned as diosgenin-3-*O*-α-L-rhamnopyranosyl(1→3)[α-L-arabinofuranosyl (1→4)]-β-D-glucopyranoside (**2a**).

Polyphyllin E (**4a**), $C_{51}H_{82}O_{20}$, 1037 (FDMS $[M+Na]^+$) mp 194–196° (decomp.), showed spiroketal ab-

*Part 4 in the series "Plant Saponins". For Part 3 see ref. [12].

Table 1. Compounds obtained after acid hydrolysis of saponins

Saponin	Wt of saponin (mg)	Diosgenin [mg (%)]	Sugars (molar ratios)
1a	15	8.2(55)	D-glucose, L-rhamnose (1:1)
2a	88.3	43.8(48)	D-glucose, L-rhamnose, L-arabinose (1:1:1)
4a	220	82.2(43.6)	D-glucose, L-rhamnose (1:3)
5a	30	12.6(42)	D-glucose, L-rhamnose (1:3)

Table 2. ^{1}H NMR spectral data of compounds **1b**, **2b**, **3b**, **4b**, **4c**, **5c** ($CDCl_3$) and **4a** (DMSO-d_6) in δ (ppm)

	1b	**2b**	**3b**	**4a**	**4b**	**4c**	**5c**
H^{I}*	4.30 (*d*, 8)	4.25 (*d*, 8)	4.32 (*d*, 8)	4.30 (*brs*, 8.5)	4.25 (*d*, 8)	4.25 (*d*, 7.5)	4.45 (*d*, 7.5)
H^{II}†	4.70 (*d*, 5)	5.10 (*d*, 5)		4.60 (*brs*, 8.5)	4.90 (*brs*, 5)	4.65 (*brs*, 5)	4.65 (*brs*, 5)
H^{III}‡		4.15 (*d*, 5)					
H^{IV}†				5.0 (*brs*, 5)	5.18 (*brs*, 5)	5.18 (*brs*, 5.5)	5.10 (*brs*, 5)
H^{V}†				5.0 (*brs*, 5)	5.18 (*brs*, 5)	5.18 (*brs*, 5.5)	5.10 (*brs*, 5)
Me(rha)	1.55 (*brs*, 8)	1.50 (*brs*, 8)		1.50 (*br*, 8)	1.50 (*brs*, 8.5)	1.50 (*brs*, 8)	1.50 (*brs*, 8)
OMe	2.8	3.28–3.45	3.25		3.2–3.5		
OAc						1.9–2.1	1.9–2.2

*H^{I}—axial anomeric H of glucose in $^4C_1$ conformation.

†H^{II}, H^{IV}, H^{V}—anomeric H of rhamnose in $^4C_1$ conformation.

‡H^{III}—axial anomeric H of arabinofuranose in F_3 conformation. The numerical values in parentheses are coupling constants in Hz.

Table 3. Molecular rotation values for saponins

Compound	$[\alpha]_D$	$[M]_D$	ΔC
Diosgenin	−129	−535	
Methyl-β-D-glucopyranoside	—	−66	
Methyl-α-L-rhanmopyranoside	—	+309	
Methyl-β-L-rhamnopyranoside	—	+170	
Methyl-α-L-rhamnopyranoside	—	−111	
Methyl-α-L-arabinofuranoside	—	−205	
Methyl-β-L-arabinoside	—	+245	
Observed for **3a**	−102	−587.5	
Calculated for **3a**	—	−601	
Observed for **1a**	−99	−714.8	
Calculated for **1a**	—	−712	
Observed for **2a**	−134	−1144.3	
Calculated for **2a**	—	−882.4	
Observed (**1a** − **3a**)			−127.3
Observed (**2a** − **1a**)	—	—	−429.5
Observed for **4a**	−97	−983.7	
Calculated for **4a**	—	−934	
Observed for **5a**	−94	−953.1	
Calculated for **5a**	—	−934	

(**1a** − **3a**): −127.3 is in agreement with α-rhamnose.

(**2a** − **1a**): −429.5 is a very high value and may not be β, the most probable configuration will be α-arabinose.

sorptions and did not give a positive Ehrlich reagent test. It was shown to be a spirostanol tetraglycoside of diosgenin by acid hydrolysis (Table 1).

The mass spectrum of the decamethyl ether (**4b**) of polyphyllin E showed prominent peaks at *m/z* 189 (trimethyl rhamnose) and 363 (trimethyl rhamnosyl dimethyl rhamnose). Kiliani hydrolysis of **4b** indicated the presence of two terminal rhamnose units and a rhamnose moiety in between glucose and rhamnose with the glucose attached at C-3 of diosgenin.

Partial hydrolysis of polyphyllin E afforded diosgenin-3-*O*-β-D-glucopyranoside (**3a**), polyphyllin C (**1a**) and PSE. The latter prosaponin on permethylation and hydrolysis furnished 2,3,4-tri-*O*-methyl-L-rhamnose, 3,4-di-*O*-methyl-L-rhamnose and 2,3,6-tri-*O*-methyl-D-glucose. Thus PSE is diosgenin-3-*O*-L-rhamnopyranosyl(1 → 2)-L-rhamnopyranosyl(1 → 4)-D-glucopyranoside.

On the basis of partial hydrolysis it is evident that one rhamnose was attached at C-3 of glucose while a birhamnosyl unit was attached to C-4 of glucose.

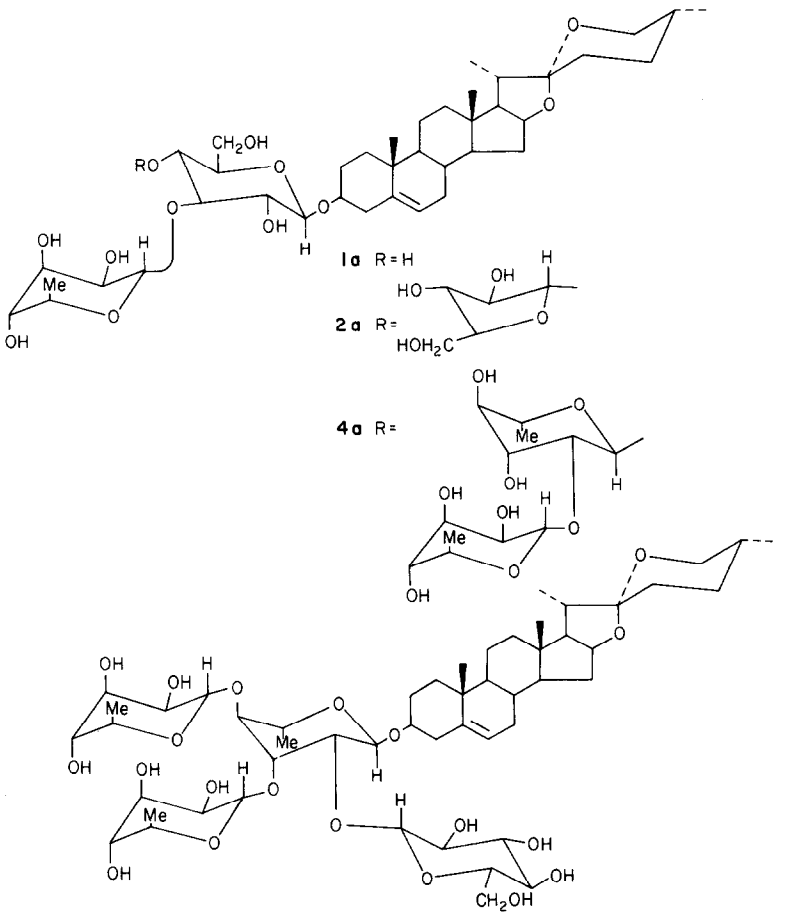
Similarly, the configuration of **4a** was assigned on the basis of the ^{1}H NMR spectrum (Table 2) and Klynes' rule (Table 3) as α to L-rhamnose and β to D-glucose. Thus the structure of polyphyllin E was assigned as diosgenin-3-*O*-α-L-rhamnopyranosyl-(1 → 2)-α-L-rhamnopyranosyl(1 → 4)[α-L-rhamnopyranosyl(1 → 3)]-β-D-glucopyranoside (**4a**).

Polyphyllin F (**5a**), $C_{51}H_{82}O_{20}$, 1053 (FDMS [M + K]$^+$) mp 190–195° (decomp.), was shown to be a spirostanol tetraglycoside of diosgenin by acid hydrolysis (Table 1), IR spiroketal absorptions and negative Ehrlich response.

Kiliani hydrolysis of the permethylate (**5b**) of polyphyllin F suggested that the C-3 diosgenin was linked to a rhamnose moiety which in turn had two rhamnose and glucose residues attached to its three hydroxyl groups through glycosidic linkages. As the partial hydrolysis of polyphyllin F afforded a prosaponin which on permethylation and hydrolysis furnished 2,3,4,6-tetra-*O*-methyl-D-glucose and 3,4-di-*O*-methyl-L-rhamnose, it is inferred that the glucose must be linked at C-2 of rhamnose.

The coupling constant values in the ^{1}H NMR spectrum of **5c** (Table 2) suggested the β-configuration for the D-glucose and α for the L-rhamnose. These assignments were also evident from application of Klynes' rule (Table 3).

Thus, the structure of polyphyllin F was assigned



as diosgenin-3-*O*-α-L-rhamnopyranosyl (1→4)[α-L-rhamnopyranosyl(1 → 3)][β-D-glucopyranosyl(1 → 2)]-α-L-rhamnopyranoside.

Seshadri and his associates [9, 10] have reported diosgenin-3-*O*-α-L-rhamnopyranosyl(1 → 4)-β-D-glucopyranoside and pariphyllin (diosgenin-3-*O*-L-rhamnopyranosyl(1 →4)[α-L-arabinofuranosyl(1 → 3)]-β-D-glucopyranoside and Kawasaki *et al.* [11] have reported diosgenin-3-*O*-α-L-rhamnopyranosyl-(1 →2)[α-L-arabinofuranosyl(1 → 4)]-β-D-glucopyranoside from tubers of *Paris polyphylla* which have been found to be different from new saponins.

The occurrence of **6a** in *P. polyphylla* leads to biogenetic speculation owing to the presence of rhamnose at C-3 of the diosgenin instead of glucose as in other known saponins. Furthermore the attachment of three sugars to one rhamnose is reported for the first time for naturally occurring saponins. This may be significant to considerations of saponin biochemistry and biosynthesis.

EXPERIMENTAL

Mps were uncorr. The IR spectra were taken in KBr pellets. The ^{1}H NMR spectra were recorded at 90 MHz in $CDCl_3$ using TMS as int. standard. Electron-impact mass spectra were recorded by direct insertion of samples into the ionization chamber, TLC was performed on Si gel (BDH) using Ehrlich reagent and 10% H_2SO_4 as visualization reagent. Descending PC was on Whatman No. 1 chromatography paper using aniline hydrogen phthalate (AHP) for staining. CC was done on Si gel (60–120 mesh BDH). The following solvent systems were used for TLC and PC. Solvent A: $CHCl_3$–MeOH–H_2O (70:35:7); solvent B: $CHCl_3$–MeOH–H_2O (60:12:2); solvent C: C_6H_6–Me_2CO (9:1); solvent D: $CHCl_3$–Me_2CO (9:1); solvent E: *n*-BuOH–AcOH–H_2O (4:1:5); solvent F: *n*-BuOH–pyridine–H_2O (6:4:3); solvent G: *n*-BuOH–EtOH–H_2O (5:1:4, upper layer).

R_G values were reported with respect to 2,3,4,6-tetra-*O*-methyl-D-glucose. The R_f values of the saponins were reported in solvent A on TLC plates (Si gel). For prep. TLC (0.75 mm) bands were detected with iodine vapour.

Isolation of saponins. Air-dried tubers (1.0 kg) collected from Nepal were defatted with boiling *n*-hexane, and later the extraction was followed by $CHCl_3$ and finally with MeOH. The MeOH extractive was concentrated. This concentrate was taken up in H_2O and extracted with *n*-BuOH (5 × 250 ml). The BuOH extract after concentration under red. pres. yielded a saponin fraction (40.6 g, 4.06%) which exhibited eight distinct spots on TLC (Solvent A), and their compounds were named as polyphyllin A–H in order of their decreasing R_f values. The saponin mixture (22.5 g) was chromatographed on Si gel (1 kg) and eluted with mixtures of $CHCl_3$–MeOH (5, 10, 15 . . . 40%). Polyphyllin A (diosgenin-3-*O*-β-D-glucopyranoside) (100 mg, 0.018%) R_f 0.70, crystals from MeOH, mp. 279–281° (decomp) $[\alpha]_D^{27}$ – 102° (pyridine, c = 0.6). (Found C, 69.1, H, 8.7; $C_{33}H_{52}O_8$ requires C, 68.8; H, 9.02%.) Polyphyllin C: (50 mg, 0.009%) R_f 0.57, crystals from MeOH. Mp 185–190° (decomp) $[\alpha]_D^{27}$ – –99° (pyridine, c = 0.5). IR ν_{max}^{KBr} cm^{-1}: 3500–3200, 1110–1000, 982, 921 (*w*), 901 (*s*), 860, 840. (Found C, 64.3; H, 8.8; $C_{39}H_{62}O_{12}$ requires C, 64.8; H, 8.58%.) MW 722.424. Polyphyllin D: (5.56 g, 1.003%) R_f 0.52, flakes from MeOH, mp 227–230° (decomp.) $[\alpha]_D^{27}$ – 134° (pyridine, c = 1.116) IR ν_{max}^{KBr} cm^{-1}: 3300; 1180–1000, 980, 920 (*w*), 900 (*s*), 860, 840. (Found C, 61.2; H, 7.95; $C_{44}H_{70}O_{16}H_2O$ requires C, 60.68; H, 8.05%.) MW 854.466. Polyphyllin E: obtained as needles from MeOH (2.52 g, 0.455%) mp 194–196° (decomp), $[\alpha]_D^{27}$ – –97° (pyridine, c = 1.09), R_f 0.45. (Found C, 59.25; H, 7.91, $C_{51}H_{82}O_{20}H_2O$ requires C, 59.30; H, 8.14.) MW 1014.540. IR ν_{max}^{KBr} cm^{-1}: 3300 (broad), 1120–1000 (broad, C–O–C), 980, 920 (*w*), 900 (*s*), 890, 840. Polyphyllin F: separation and crystallization of this compound was difficult, hence the mixture was acetylated with Ac_2O–pyridine and polyphyllin F was obtained after alkaline hydrolysis, as crystals from MeOH (100 mg) mp 190–195° (decomp.) $[\alpha]_D^{27}$ – 94° (pyridine, c = 0.5), R_f 0.40. (Found C, 57.92; H, 7.95, $C_{51}H_{82}O_{20}2H_2O$ requires C, 58.28; H, 8.19.) MW 1014.540. IR ν_{max}^{KBr} cm^{-1}: 3400 (broad), 1150–1000 (C–O–C), 980, 925 (*w*), 905 (*s*), 880, 840. Other saponins also were isolated with increasing polarity of the eluent. (The percentage of the compound obtained is based on crystalline compounds per dry wt of plant material.)

Acid hydrolysis of saponins. All the saponins were hydrolysed separately as described earlier [12]. The common genin in all compounds was identified as diosgenin by comparison (mmp, co-TLC, IR, ^{1}H NMR and MS) with an authentic sample and sugars were identified by co-PC (solvents E and F). The estimation of the sugars was performed by the colorimetric method [8, 13]. The results of hydrolysis are presented in Table 1.

Permethylation of saponins. Permethylation of all saponins was done separately by the method of refs. [12, 14]. IR spectra of individual permethylates were checked for hydroxyl bands which were found to be absent. Compound **1a** (25 mg) yielded **2b** (20 mg) a hexamethyl ether, syrup, R_f, 0.53 (solvent C). Compound **2a** (300 mg) furnished the octamethyl ether (**2b**, 250 mg), amorphous powder, mp 143–145°, R_f 0.38 (solvent C); MS *m/z* (rel. int.): 189 (44.4), 175 (45.7), 141 (5.9), 139 (6.1), 115 (3.4), 57 (100). Compound **4a** (200 mg) afforded a decamethyl ether (**4b**, 150 mg), amorphous powder, mp 88–90°, R_f 0.35 (solvent C); MS *m/z* (rel. int.): 363 (15.5), 279 (11.1), 189 (15.1), 167 (27.9), 157 (18.9), 156 (54.6), 149 (100), 141 (50.1), 139 (6.0), 115 (9.9). Compound **5a** (30 mg) furnished a decamethyl ether (**5b**, 25 mg), homogeneous syrup, R_f 0.32 (solvent C).

Kiliani hydrolysis of permethylates. The permethylated saponins were hydrolysed by Kiliani mixture (AcOH, HCl, H_2O, 1.5, 3.5, 5) by a procedure described earlier [12]. The methylated sugars thus obtained were identified by their R_G values (solvent G) [**4b** 10, 15] and direct comparison with authentic samples on PC. The R_G values of the hydrolysis products were as follows; **1b**: 1.01, 0.74; **2b**: 1.01, 0.97, 0.64; **3b**: 1.00; **4b**: 1.01, 0.84, 0.64; **5b**: 1.01, 1.00, 0.30. The identities of the compounds based upon R_G value are: 1.01 = 2,3,4-tri-*O*-methyl-L-rhamnose; 0.97 = 2,3,5-tri-*O*-methyl-L-arabinose; 0.84 = 3,4-di-*O*-methyl-L-rhamnose; 0.74 = 2,4,6-tri-*O*-D-glucose; 0.64 = 2,6-di-*O*-methyl-D-glucose (confirmed by periodate oxidation); 0.30 = L-rhamnose.

Periodic acid oxidation of methylated sugars. The methylated sugars **2b** and **4b** (10 mg each) were stirred with HIO_4 (0.5 ml) for 4 hr. The reaction mixture (DNP positive) was directly examined for methylated sugars on PC (solvent G) which showed only two spots in each case corresponding to R_G 1.01, 0.97 and 1.01, 0.84, respectively. This experiment confirmed the presence of a vicinal diol group in one of the methylated sugars (R_G 0.64) thus confirming this methylated sugar as 2,6-di-*O*-methyl-D-glucose and not 2,4-di-*O*-methyl-D-glucose.

Smith degradation of polyphyllin C and D. Compound **1a** (250 mg) in aq. MeOH (20 ml) was added to $NaIO_4$ (275 mg)

and stirred at room temp. for 6 hr until oxidation was complete (TLC monitored). A faster-moving compound was detected (DNP spray reagent) on TLC (solvent B) from the reaction mixture which was diluted with H_2O and extracted with *n*-BuOH. The organic layer was washed with H_2O and solvent was removed *in vacuo*. The residue was dissolved in MeOH (15 ml) and $NaBH_4$ (150 mg) was added and the mixture stirred at room temp. for 4 hr. The reaction mixture was diluted with H_2O and acidified to pH 1.8–2.0 with 2 N H_2SO_4. The acidic soln was kept at room temp. for 48 hr with occasional shaking. This reaction mixture was extracted with *n*-BuOH and afforded a prosaponin which was identified as diosgenin 3-*O*-β-D-glucopyranoside by comparison with an authentic sample and of its permethylate (**3b**) (hydrolysis) and peracetates (**3c**), IR, MS, NMR. Similarly polyphyllin C (5 mg) was subjected to Smith degradation which also afforded **4a**.

Partial hydrolysis of polyphyllins C, D, E and F. Polyphyllin C (5 mg) was refluxed with 1% MeOH–H_2SO_4 (5 ml) for 25 min. The reaction mixture was immediately diluted with H_2O and extracted with *n*-BuOH. The *n*-BuOH layers afforded a prosaponin **3a**. Polyphyllin D (500 mg) on partial hydrolysis furnished prosaponins **2a** and **4a** which were purified by prep. TLC (solvent B).

Polyphyllin E (200 mg) was subjected to a similar partial hydrolysis and after separation on prep. TLC (solvent B) and elution with ($CHCl_3$–MeOH, 70:30) furnished **3a** (30 mg) 279–281° (decomp.), **1a** (40 mg) 185–188° (decomp.) and PSE (20 mg) 205–210° (decomp.) in decreasing R_f values.

PSE on permethylation employing the above method furnished an octamethyl ether (as syrup) which on Kiliani hydrolysis afforded methylated sugars R_G 1.01, 0.84 and 0.83 identified as 2,3,4-tri-*O*-methyl-L-rhamnose, 3,4-di-*O*-methyl-L-rhamnose and 2,3,6-tri-*O*-methyl-D-glucose (co–PC, solvent G).

Compound **5a** (25 mg) was similarly partially hydrolysed to afford a prosaponin (5 mg) which on methylation by the method described above and Kiliani hydrolysis afforded methylated sugars with R_G 1.00 and 0.84 identified as 2,3,4,6-tetra-*O*-methyl-D-glucose and 3,4-di-*O*-methyl-L-rhamnose (co–PC, solvent G).

Acetate of saponin acetates. Compound **2a** (300 mg) furnished an octa-acetate **2b** (281 mg) crystallized from hexane–Et_2O mp 113–117°; R_f 0.74, (solvent D). IR ν_{max}^{KBr} cm^{-1}: 1750, 1230 (MeCOO). MS *m/z* (rel. int.): 413 (5.5), 412 (11.1), 273 (68.63), 259 (53.90), 141 (83.8), 139 (100), 115 (28.11). Acetate (**3c**) of **3a**, amorphous powder, mp 195–197°, IR ν_{max}^{KBr} cm^{-1}: 1735, 1220 (MeCOO). MS *m/z* (rel. int.): 744 $[M]^+$ (1.2), 413 (11.9), 412 (22.4), 397 (22.58), 396 (23.34), 331 (10.93), 141 (28.3), 139 (73.82), 43 (100). Deca-acetate, **4c**, amorphous powder, mp 92–95°, IR ν_{max}^{KBr} cm^{-1}: 1740, 1220 (MeCOO), 980, 920 (*w*), 900 (*s*), 880, 840.

The mixture of the later chromatographic fractions of polyphyllin E and F (500 mg) was acetylated. The mixture of acetates of E and F was purified on a Si gel column by eluting with solvent C to afford the deca-acetate of polyphyllin F (200 mg) as an amorphous powder, mp 135–138°, IR ν_{max}^{KBr} cm^{-1}: 1740, 1220 ($COOCH_3$), 980, 915 (*w*), 900 (*s*), 880, 840.

Alkaline hydrolysis of **5c**. Compound **5c** (180 mg) was heated at 50° with 5% methanolic KOH soln for 2 hr. The reaction mixture when extracted with *n*-BuOH afforded polyphyllin F (100 mg) crystallized from MeOH.

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