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FUROSTANOL SAPONINS FROM *PARIS POLYPHYLLA*: STRUCTURES OF POLYPHYLLIN G AND H*

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Key Word Index—*Paris polyphylla*; Liliaceae; diosgenin; saponin; polyphyllin G; 3-*O*-{α-L-rhamnopyranosyl (1→3) [α-L-arabinofuranosyl (1→4)]-β-D-glucopyranosyl}-26-*O*-[β-D-glucopyranosyl] (25*R*)-22α-hydroxy-furost-5-en-3β, 26-diol; polyphyllin H; 22-methoxy-polyphyllin G.

Abstract—The structure of two new saponins, polyphyllins G and H, isolated from the tubers of *Paris polyphylla* have been elucidated as 3-*O*-{α-L-rhamnopyranosyl (1→3) [α-L-arabinofuranosyl (1→4)]-β-D-glucopyranosyl}-26-*O*-[β-D-glucopyranosyl] (25*R*)-22α-hydroxy-furost-5-en-3β, 26-diol and its 22-methoxy derivative respectively.

INTRODUCTION

In our previous communications [1, 2] we reported the isolation and structure of spirostanol saponins from *Paris polyphylla*. Here, we report the structure of two new furostanol saponins isolated for the first time from *P. polyphylla* tuber.

RESULTS AND DISCUSSION

Polyphyllin G (**1a**), $C_{51}H_{84}O_{22}$, *m/z* 1071 (M + Na, FDMS) mp 177–181° (decomp.) $[\alpha]_D^{27} - 76.9°$ (pyridine; *c* 0.13) $[M]_D^{27} - 805.9$ exhibited a pink spot with Ehrlich reagent [3] and absorbed at 970, 920(s), 900(w) and 880 cm^{-1} in the IR spectrum [4] which is characteristic of a furostanol structure.

Complete acid hydrolysis of **1a** afforded the genin, which was identified as diosgenin (**3**) (TLC, mmp, IR, NMR and MS), and D-glucose, L-rhamnose and L-arabinose. The quantitative assay of the sugars D-glucose, L-rhamnose and L-arabinose showed them to be in the molar ratios (2:1:1) [5, 6]. Acetylation of **1a** afforded the dodeca-acetate (**1b**) with a ^1H NMR signal at δ 3.58 (3H, *s*) attributable to methoxy protons. The probable location of a methoxy group in a furostanol glycoside appears to be at C-22.

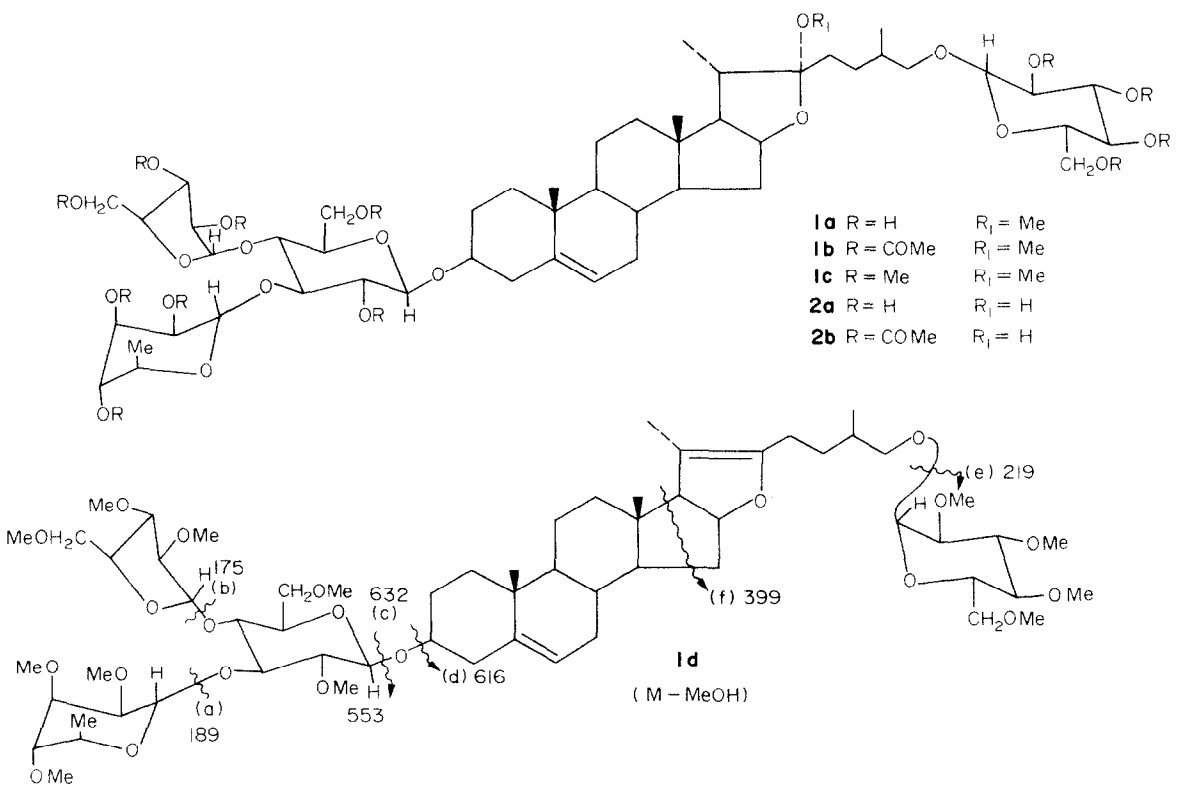
Polyphyllin G on permethylation [7] furnished the dodecamethyl ether (**1c**) which lacked an $[M]^+$ ion in the mass spectrum. The loss of methanol in the mass spectrum of 22-methoxy furostanol glycosides [8] is a very facile process. Therefore, the general fragmentation takes place from **1d**, a comparatively stable molecule in mass spectrometry. Compound **1d** $[M-32]^+$ showed fragments at *m/z* 219, 189 and 175 by cleavage at *e*, *a*, *b*, corresponding to tetramethyl glucose, trimethyl rhamnose and trimethyl arabinose respectively. The ion obtained at *m/z* 553 (*c*) was due to the formation of a single oxonium ion of the three permethylated sugars with uneven mass number. The formation of ions at *m/z* 632, 616 and 399 under hydrogen migration could be explained as $[c + H]^+$, $[d + H]^+$ and $[f + H]^+$ in **1d** respectively. On the basis of the above mass spectral data it was concluded that arabinose and rhamnose are attached to glucose as terminal sugars at C-3 of diosgenin and the other glucose is attached at C-26.

Kiliani hydrolysis of **1c** furnished four methylated sugars with R_G (PC) 1.01, 1.00, 0.97 and 0.64 identified as 2, 3, 4-tri-*O*-methyl-L-rhamnose, 2, 3, 4, 6-tetra-*O*-methyl-D-glucose, 2,3,5-tri-*O*-methyl-L-arabinose and 2,6-di-*O*-methyl-D-glucose respectively [9, 10]. The identities of all the methylated sugars were also confirmed by comparison with their authentic samples.

The inter-sugar linkages of rhamnose and arabinose with glucose have been shown by partial and enzymatic hydrolysis of polyphyllin G. Compound **1a** on partial hydrolysis afforded the prosaponins **4a** and **5a** in decreasing order of R_f values (**4a** and **5a** were Ehrlich negative). Compound **4a** on hydrolysis furnished diosgenin and D-glucose and on permethylation and hydrolysis afforded diosgenin and 2, 3, 4, 6-tetra-*O*-methyl-D-glucose and therefore, it was identified as diosgenin-3-*O*-β-D-glucopyranoside (polyphyllin A). On hydrolysis compound **5a** afforded diosgenin, D-glucose and L-rhamnose (1:1) and on permethylation and hydrolysis afforded diosgenin, 2, 3, 4-tri-*O*-methyl-L-rhamnose (R_G 1.01) and 2, 4, 6-tri-*O*-methyl-D-glucose (R_G 0.76). Therefore, the structure for **5a** was assigned as diosgenin 3-*O*-α-L-rhamnopyranosyl (1→3)-β-D-glucopyranoside, which was identical with polyphyllin C (TLC, mmp, IR).

Compound **1a** on enzymatic hydrolysis with almond emulsin afforded a glycoside which was identical with polyphyllin D (diosgenin-3-*O*-α-L-rham-

*Part V in the series "Plant Saponins". For Part IV see Singh, S. B. and Thakur, R. S. (1982) *Phytochemistry* (in press).



nopyranosyl (1→3) [α-L-arabinofuranosyl (1→4)]-β-D-glucopyranoside).

The ^{1}H NMR spectrum of **1c** displayed signals at δ 4.05 (1H, *d*, *J* = 7.5 Hz, H-1 axial in $^4C_1$ conformation of glucose at C-26) 4.12 (1H, *d*, *J* = 4.5 Hz, H-1 axial in E_3 conformation of arabinose), 4.25 (1H, *d*, *J* = 8 Hz, H-1 axial of glucose) and at 5.10 (1H, *d*, *J* = 5.5 Hz, H-1 axial in $^4C_1$ conformation of rhamnose) for β-glucose, α-arabinose, β-glucose and α-rhamnose respectively. ^{1}H NMR values were compared with the permethylate of polyphyllin D). The Klynes rule [11] of molecular rotation also supported the above configuration for the sugars.

On the basis of the above data the structure for polyphyllin G has been assigned as 3-*O*-{α-L-rhamnopyranosyl (1→3) [α-L-arabinofuranosyl (1→4)]-β-D-glucopyranosyl}-26-*O*-[β-D-glucopyranosyl]-(25*R*)-22α-methoxy-furost-5-en-3β, 26-diol.

The isolation of polyphyllin H in the pure state was difficult due to its high polarity and very low concentration. Therefore, it was isolated after acetylation and subsequent alkaline hydrolysis of the acetate. The ^{1}H NMR spectra of both the acetates, i.e. acetates of polyphyllin G (**1b**) and polyphyllin H (**2b**) were almost superimposable except at the region δ 3.58. In the ^{1}H NMR spectrum of the latter compound, a signal due to methoxyl protons was lacking while it was present in the former. The IR spectrum of **2b** had a broad absorption band at 3500 cm^{-1} for a hydroxyl function. The alkaline hydrolysis of **2b** afforded polyphyllin H (**2a**) which on boiling with methanol was converted into **1a**.

Polyphyllin G on boiling with aqueous acetone yielded **2a**. This type of interconversion of 22-hydroxy into 22-methoxy and *vice versa* is known [12]. Therefore, it has been concluded that the polyphyllin G was the corresponding methyl ether of the 22-hydroxy compound polyphyllin H. Hence, the structure for **2a** has been arrived at as 3-*O*-{α-L-rhamnopyranosyl (1→3) [α-L-arabinofuranosyl (1→4)]-β-D-glucopyranosyl}-26-*O*-[β-D-glucopyranosyl]-(25*R*)-furost-5-en-3β, 22α, 26-triol.

EXPERIMENTAL

Mps were uncorr. The IR spectra were taken in KBr pellets. The ^{1}H NMR spectra were recorded in $CDCl_3$ using TMS as int. standard. Electron-impact MS were recorded by direct insertion of samples in to the ionization chamber. The field desorption (FD) mass spectra were obtained under the experimental conditions as reported previously [13, 14]. TLC was performed on Si gel using Ehrlich reagent and 10% H_2SO_4 as detection agent. Descending PC was on Whatmann No. 1 chromatography paper using aniline hydrogen phthalate (AHP) for staining. CC was on Si gel (60–120 mesh). The following solvent systems were used for TLC and PC: (A) $CHCl_3$–MeOH–H_2O (70:35:7); (B) $CHCl_3$–MeOH–H_2O (60:12:2); (C) C_6H_6–Me_2CO (9:1); (D) C_6H_6–pyridine–H_2O (6:4:3); (G) *n*-BuOH–EtOH–H_2O (5:1:4, upper layer). R_G values were reported with respect to 2, 3, 4, 6-tetramethyl glucose. The R_f values of the saponins were reported in solvent A on Si gel TLC. For PLC, plates were 0.75 mm thick and bands were detected in an iodine chamber.

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 concd and taken up in H_2O and extracted with *n*-BuOH (5 × 250 ml). The BuOH extract after concn under red. pres. yielded the saponin fraction (40.6 g, 4.06%) which exhibited eight distinct spots

Table 1. Calculated and observed $[M]_D$ values for polyphyllin G

Compound	$[\alpha]_D$	$[M]_D$
Diosgenin	−129	−535
Methyl-β-D-glucopyranoside	—	−66
Methyl-α-L-rhamnopyranoside	—	−111
Methyl-α-L-arabinofuranoside	—	−205
Observed for **1a**	−76.9	−805.91
Calculated for **1a**	—	−948.4

The following methylated sugars have also been considered for the calculation of $[M]_D$ values for other possible combinations: methyl-α-D-glucopyranoside, +309; methyl-β-L-rhamnopyranoside, +170; and methyl-β-L-arabinoside, +245.

on TLC (solvent A) which were named as polyphyllins A–H in order of their decreasing R_f values. The saponin mixture (22.5 g) was chromatographed on a Si gel (1 kg.) column and eluted with mixtures of $CHCl_3$ and MeOH (5, 10, . . . , 40%). Polyphyllin A and C–F were obtained in the early fractions of 15% MeOH, but latter fractions on crystallization afforded polyphyllin G (500 mg, 0.09%) MW 1048.545 (FD–MS) mp 177–181° (decomp.). R_f 0.36 $[\alpha]_D^{27}$ − 76.9° (pyridine; *c* 0.13) $[M]_D^{27}$ − 805.91, (found C, 56.2, H, 7.95. $C_{51}H_{84}O_{22}$ $2H_2O$ requires C, 56.45, H, 8.12%.) IR ν_{max}^{KBr} cm^{-1}: 3500–3100 (OH), 1150–1000 (C–O–C), 970, 920(s), 900(w), 880. The 20% MeOH fractions yielded a mixture of polyphyllins G and H (2 g).

Hydrolysis of polyphyllin G. Polyphyllin G (25.9 mg) was refluxed with 7% methanolic H_2SO_4 (20 ml) for 7 hr. The reaction mixture was diluted with H_2O, MeOH was removed and the residue extracted with $CHCl_3$. The organic layer was washed with H_2O, dried over Na_2SO_4 and $CHCl_3$ removed under red. pres. The crude solid (11.6 mg, 41%) upon crystallization yielded the aglycone, mp 201–203°, M^+ at *m/z* 414 which was identified as diosgenin (TLC, mmp, IR, NMR, MS). The aq. soln was neutralized by passing through Dowex-1^- (OH^- form), H_2O was removed under vacuum and L-rhamnose, D-glucose and L-arabinose were obtained from the hydrolysate (PC, solvent E and F). The molar ratio of the sugars were determined by a colorimetric method of estimation and was found to be 2:1:1.

Partial hydrolysis of polyphillin G. Compound **1a** (100 mg) was refluxed with 1% MeOH–H_2SO_4 for 35 min. The reaction mixture was diluted with H_2O and extracted with *n*-BuOH. The *n*-BuOH layer after washing with H_2O and removal of the organic solvent under vacuum yielded a mixture of prosaponins which were separated on PLC (solvent B), **4a** (20 mg) mp 278–280° (decomp.) $[\alpha]_D^{27}$ − 102° (pyridine) and **5a**, (15 mg), mp 185–190° (decomp.) $[\alpha]_D^{25}$ − 99° (pyridine). The former was identical with polyphillin A while the latter was polyphyllin C (TLC, mmp).

Permethylation of polyphyllin G. Polyphyllin G (230 mg) in DMSO (15 ml) was added dropwise to a stirred soln of 50% NaH (1.4 g, washed with *n*-hexane) and DMSO (60 ml) in an oil bath at 70° until a green colour appeared (3 hr). This reaction mixture was cooled and MeI (15 ml) was added drop-wise and the reaction mixture kept under continuous stirring overnight. After dilution with ice-cold H_2O it was extracted with Et_2O (5 × 200 ml). The Et_2O layer was washed with H_2O, dried over Na_2SO_4 and distilled. The crude product (190 mg) on PLC (solvent C) afforded the dodecamethyl ether of polyphyllin G (**1a**) crystallized from hexane–Et_2O (125 ml), mp 107–110° R_f 0.3 (solvent C). ^1H NMR ($CDCl_3$) δ 1.47 (3H, *br s*, $W_{1/2}$ = 8 Hz, $-CH_3$ of rhamnose,) MS *m/z* (rel. int.): 632 (4.1), 616 (4.1), 553 (4.2), 435 (43.6), 413 (16.2), 399 (4.7), 397 (24.2), 396 (10.9), 395 (44.5), 299 (25.0), 219 (36.5), 189 (88.5), 187 (38.1), 175 (37.8), 157 (30.2), 111 (27.6), 101 (100), 88 (98).

Hydrolysis of **1c**. Compound **1c** (50 mg) was hydrolysed with Kiliani mixture (5 ml). The reaction mixture on usual work-up afforded methylated sugars which on PC (solvent G), revealed the presence of four methylated sugars corresponding to R_G 1.01, 1.00, 0.97 and 0.64 and these were identified as 2, 3, 4-tri-*O*-methyl-L-rhamnose 2, 3, 4, 6-tetra-*O*-methyl-D-glucose, 2, 3, 5-tri-*O*-methyl-L-arabinose and 2, 6, di-*O*-methyl-D-glucose, respectively. These identifications were confirmed by comparison with authentic samples.

Periodic acid oxidation of methylated sugars. A part of the methylated sugars (from the previous experiment) was oxidized with HIO_4 (0.5 ml) by stirring at room temp. for 4 hr. After work-up the DNP-positive reaction mixture on PC showed only three out of the previous total of four spots for methylated sugars of R_T 1.01, 1.00 and 0.97 (see below for previous expt). This confirmed the presence of a methylated sugar containing a vicinal dihydroxy group, i.e. 2, 6-di-*O*-methyl-D-glucose.

Enzymatic hydrolysis of polyphyllin G. Polyphyllin G (100 mg) was incubated with almond emulsin for 7 days at 37°. The reaction mixture was extracted with *n*-BuOH after dilution with H_2O. The *n*-BuOH extract after usual work-up and PLC (solvent B) afforded polyphyllin D (30 mg) mp 227–230° (decomp.).

Acetylation of polyphyllin G. The mother liquor of the chromatographic fractions of polyphyllin G (300 mg) was acetylated with Ac_2O–pyridine (5 ml). PLC (solvent D) afforded the dodecacetate of polyphyllin G (100 mg), mp 145°, R_f 0.60 (solvent D). IR ν_{max}^{KBr} cm^{-1}: 1750, 1230 (ester). ^1H NMR (60 MHz, $CDCl_3$): δ 1.9–2.2 (36 H, 12 × $-COCH_3$) 3.58 (3H, *s*, OMe).

Acetylation of polyphyllin H. The latter fractions of the 20% MeOH eluate of the chromatography of saponins (2.0 g) were acetylated with Ac_2O–pyridine (15 ml) to afford a mixture of acetates which on chromatography on Si gel and elution with C_6H_6–Me_2CO (85:15) furnished the dodeca-acetate of polyphyllin H (**2b**) (100 mg) as an amorphous powder mp 110–111°, R_f 0.50 (solvent D). IR ν_{max}^{KBr} cm^{-1}: 3500 (OH), 1750, 1230 (COOMe), ^1H NMR (60 MHz, $CDCl_3$): δ 1.9–2.2 (36H, 12 × $COCH_3$).

Alkaline hydrolysis of the acetate of polyphyllin H. Compound **2b** (50 mg) was heated at 50° with 5% methanolic KOH soln (20 ml) for 2 hr. The soln was cooled to room temp. diluted with H_2O and acidified with dil. HCl to pH 7 and extracted with *n*-BuOH. The *n*-BuOH layer, after usual work-up, was chromatographed over Si gel (10 g) and eluted with $CHCl_3$–MeOH (85:15). Polyphyllin H (20 mg) was obtained as a gummy material as crystallization was difficult, R_f 0.32.

Hydrolysis of polyphyllin H. Polyphyllin H (10 mg) was hydrolysed with 7% MeOH–H_2SO_4. It afforded diosgenin (TLC) and D-glucose, L-rhamnose and L-arabinose (2:1:1) (colorimetric estimation).

Conversion of polyphyllin H to G and vice versa. Polyphyllin H (10 mg) was refluxed with MeOH (100 ml) for 6 hr and afforded polyphyllin G (10 mg) which on boiling with aq. Me_2CO (100 ml) over a water bath for 6 hr yielded polyphyllin H.

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