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STRUCTURE CHARACTERIZATION OF HAEMOSTATIC DIOSGENIN GLYCOSIDES FROM *PARIS POLYPHYLIA*

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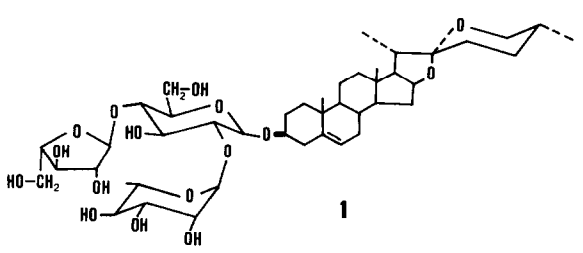
Key Word Index—*Paris polyphylla*; Liliaceae; polyphyllin D; pariphyllin; yunnan paiyao, haemostatic and cytotoxic diosgenin glycosides

Abstract—From *Paris polyphylla* var. *chinensis* Hare (Liliaceae), four diosgenin glycosides with haemostatic effects were isolated. The structure of the major component was elucidated by chemical and spectroscopic methods as 3-{[α-L-rhamnopyranosyl($1_{Rha} \rightarrow 2_{Glu}$)]-[α-L-arabinofuranosyl($1_{Ara} \rightarrow 4_{Glu}$)]-β-D-glucopyranosyl}-25(*R*)-spirost-5-en-3β-ol. This saponin was found to be identical to three previously reported compounds to which other structures were originally assigned, namely the major component from *P. polyphylla* Smith, the major cytotoxic component of yunnan paiyao, and polyphyllin D from *P. polyphylla* grown in the Himalaya region.

INTRODUCTION

From *Paris polyphylla* var. *chinensis* Hare, a haemostatic glycoside of diosgenin, $C_{44}H_{70}O_{16}$ (**1**), mp 275–280° was isolated in our laboratory. The structure of saponin (**1**) was elucidated by chemical and spectroscopic methods as a glycoside of diosgenin with an arabinose attached to the C-4 and a rhamnose to the C-2 position of glucose. In the lit., four different compounds similar to saponin (**1**) have been reported: (i) a trisaccharide glycoside isolated from *P. polyphylla* Smith [1–3] having two more hydrogens than **1** in the assigned molecular formula ($C_{44}H_{72}O_{16}$); (ii) a cytotoxic component from yunnan paiyao† [4], with the same structure as **1** but having a much lower mp (242–248°); (iii) the major component of *P. polyphylla* grown in the Himalaya region, named polyphyllin D, whose structure was reported to be similar to **1** except that the linkages of arabinose and rhamnose were placed at positions C-4 and C-3 of glucose, respectively [5]; and (iv) another component from the same Himalaya species with similar structure except the linkages of arabinose and rhamnose were given at positions C-3 and C-4 of glucose, respectively [6]. In order to clarify the inconsistencies in the lit., a full investigation of the structure of saponin (**1**) was conducted.



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†A formulated Chinese folk medicine to stop bleeding.

RESULTS AND DISCUSSION

Four polysaccharide diosgenin glycosides, **1** ($C_{44}H_{70}O_{16}$), **2** ($C_{45}H_{72}O_{16}$), **3** ($C_{45}H_{72}O_{17}$) and **4** ($C_{51}H_{82}O_{20}$) were isolated from *P. polyphylla* var. *chinensis* Hare. After hydrolysis, saponin (**1**) yielded arabinose, rhamnose, glucose and diosgenin. From fast atom bombardment mass spectrometry (FABMS), saponin (**1**) was elucidated as Ara–(Rha–Glu)-diosgenin, in which the first sugar was the terminal group. Similarly, **2**–**4** were assigned as Rha–(Rha–Glu)-diosgenin, Glu–(Rha–Glu)-diosgenin and Rha–(Rha_2–Glu)-diosgenin, respectively. The assignments were confirmed by the hydrolysis products.

In the partial hydrolysis of **1**, trillin (or Glu-diosgenin) and Rha–Glu-diosgenin (**5**) were obtained to indicate that the C-1 position of glucose is linked to the 3β-position of diosgenin. Permethylation of **5** followed by methanolysis yielded **6**, which was identified as methyl-3,4,6-trimethoxyglucoside by GC/MS (Table 1 [7]). The linkage between glucose and rhamnose should, therefore, occur at the C-2 position of the former.

Saponin (**1**) was permethylated to produce **7**, which yielded **8** after methanolysis. The mass spectrum of **8** was found to be similar to both methyl-3,6-dimethoxyglucoside and methyl-2,6-dimethoxyglucoside as listed in Table 2 [7]. But the mass spectrum of TMSi-**8** ruled out the possibility of **8** being a 2,6-homologue because of the presence of the peak at *m/z* 217. Therefore, the two hydroxyl groups in the glucose should be located at positions C-2 and C-4. Since the C-2 position of **1** is already linked to rhamnose, the remaining C-4 position must be occupied by arabinose.

The *R*-spiroketal configuration at the C-25 position was confirmed by the IR spectrum of **1** (intensity of 900 cm^{-1} > 920 cm^{-1}) and the ^{13}C NMR spectrum of the hydrolysis product, diosgenin (δ30.4) [8].

The difference between diosgenin and trillin molecular rotations was −38°, which is in good agreement with the calculated Δ [φ] for methyl-β-D-glucopyranoside. The Δ [φ] between trillin and **5** was −142° indicating that the

Table 1 Mass spectra of methyl tri-*O*-methyl glucosides and their *O*-TMSi-derivatives [7]

Methyl glucosides	*m/z*														
	45	71	73	74	75	87	88	89	101	102	133	146	159	161	187
2,3,6-Methoxy	45*	—	—	13	83	15	100	—	16	—	—	—	22	16	15
3,4,6-Methoxy	54	100	17	63	94	23	59	20	25	11	—	—	4	13	—
6	43	100	17	56	95	23	64	20	28	12	—	—	3	11	—
TMSi-2,3,6-methoxy	35	—	44	7	55	3	100	25	25	—	12	14	89	—	—
TMSi-3,4,6-methoxy	66	43	66	15	52	4	28	41	19	6	12	100	78	7	—
TMSi-**6**	28	32	54	9	61	3	29	29	15	4	14	100	98	6	2

*Relative intensity of ion.

Table 2. Mass spectra of methyl di-*O*-methyl glucosides and their *O*-TMSi-derivatives [7]

Methyl glucosides	*m/z*														
	45	71	73	74	75	87	88	89	101	102	133	146	147	159	217
2,6-Methoxy	25*	—	5	100	8	43	2	9	2	—	—	—	—	—	—
3,6-Methoxy	66	—	7	100	65	61	—	5	2	—	—	—	—	—	—
8	43	20	7	100	74	22	5	3	5	—	—	—	3	5	—
8-Nepal	43	19	7	100	58	21	5	3	5	—	—	—	1	5	—
TMSi-2,6-methoxy	16	—	51	5	9	1	5	15	3	—	32	100	—	22	1
TMSi-3,6-methoxy	20	—	60	6	26	1	1	33	5	3	—	88	—	13	100
TMSi-**8**	13	3	53	7	44	2	1	26	5	—	7	99	16	21	100
TMSi-**8**-Nepal	15	3	71	8	22	2	—	28	4	—	8	100	28	19	98

*Relative intensity of ion.

rhamnose can be either α-L-rhamnopyranose or α-L-rhamnofuranose; the ^{13}C NMR spectrum of **1** was in good agreement with the pyranosyl form of the rhamnose moiety [9]. Similarly, the Δ [ϕ] of −258° between **5** and **1** indicated an α-L-arabinofuranose.

The structure of **1** was then elucidated to be 3-{[α-L-rhamnopyranosyl($1_{Rha} \rightarrow 2_{Glu}$)]-[α-L-arabinofuranosyl($1_{Ara} \rightarrow 4_{Glu}$)]-β-D-glucopyranosyl}-25(*R*)-spirost-5-en-3β-ol. The structure was found to be identical to the major component of *P. polyphylla* whose molecular formula ($C_{44}H_{72}O_{16}$), as previously reported by Noharo *et al.* [1], Chen and Zhou [2] and Lay and Chiang [3], has two more hydrogens than **1**. The correct formula should be $C_{44}H_{70}O_{16}$ as indicated by the M_r of 854 measured by FABMS (*m/z*: 855 $[M+H]^+$). In the ^{13}C NMR spectrum, the double bond in **1** was confirmed by the peaks at δ140.9 and 122.2 assigned for C-5 and C-6, respectively, and the double bond in the hydrolysis product diosgenin was in agreement with the published data [8].

A second compound similar to saponin (**1**) was isolated from yunnan paiyao by Ravikumar *et al.* [4] with an mp some 30° lower than **1**. When we repeated our extraction, isolation and purification procedure on yunnan paiyao, the major saponin isolated was found to be completely identical to **1** in mmp, R_f values in two solvent systems (A and B), IR, ^1H NMR, ^{13}C NMR and mass spectra. In the purification of **1**, the chromatographic fractions containing only single spots were used for re-crystallization to produce the high mp sample of **1**. On the other hand, Sih divided his column chromatographed extract into two fractions, and used the first half for re-crystallization to produce the low mp material. When we tried to use a less pure chromatographed material for re-crystallization, a low mp product was obtained which proved to be a mixture of **1** and its homologue containing one extra oxygen in the aglycone. From FABMS analysis of the mixture, after substraction of **1**, the following positive ion fragments were obtained at *m/z* (rel. int.): 871 $[M+H]^+$ ($C_{44}H_{70}O_{17}$) (15), 739 $[871-Ara+H_2O]^+$ (13), 593 $[739-Rha+H_2O]^+$ (7), 431 $[593-Glu+H_2O]^+$ (28) and 413 $[431-H_2O]^+$ (100). Negative ion fragments were seen at *m/z*: 869 (100), 737 (54), 591 (10), 429 (10) and 411(13). Apparently, the impurity in the low mp sample could possibly be 3-{[α-L-rhamnopyranosyl($1_{Rha} \rightarrow 2_{Glu}$)]-[α-L-arabinofuranosyl($1_{Ara} \rightarrow 4_{Glu}$)]-β-D-glucopyranosyl}-25(*R*)-spirost-5-en-3β,17α-diol (**9***) [10]. Since the structure of the low mp sample is completely identical to **1**, the correct mp should presumably be 275–280°, instead of 242–248° as previously reported.

Singh *et al.* [5] isolated several glycosides from *Paris*

*The correct molecular formula of **9** should be $C_{44}H_{70}O_{17}$ instead of $C_{44}H_{72}O_{17}$ as reported by Miyamura *et al.* [10] and Chen *et al* [11]

Table 3. R_G values of methylated glucoses

Name of compound	*n*-BuOH–EtOH–NH_3–H_2O (40:10:1:49, top layer)		*n*-BuOH–EtOH–H_2O (50:10:40, top layer) (solvent system C)	
	Hirst [14]	Rafique [15]	Hirst [13]	Ma and Lau, this work
Glucose	0.09	—	0.09	0 18
3-Methoxyglucose	0.27	0.40	0.26	0.38
2,3-Dimethoxyglucose	0.57	0.68	0 57	—
2,3,6-Trimethoxyglucose	0.81	0.88	0.83	—
2,3,4,6-Tetramethoxyglucose	1.00	1.00	1 00	1.00
3,6-Dimethoxyglucose	0.51	—	0 51	—
or **10**	—	—	—	0.61
10-Nepal	—	—	—	0.61

polyphylla grown in the Himalaya region and proposed the structure of the major component Polyphyllin D (mp 227–230°) to be $Rha_1 \rightarrow {}_3(Ara_1 \rightarrow {}_4Glu)$-diosgenin (**C**). Seshadri and Vydeeswaran [6] isolated a trisaccharide glycoside of diosgenin from the same species with the proposed structure $Rha_1 \rightarrow {}_4(Ara_1 \rightarrow {}_3Glu)$-diosgenin (mp 294–298°) and named it pariphyllin. Their reasoning was based on the R_G value of the hydrolysis product ('X') from permethylated pariphyllin and polyphyllin D. The R_G value of this hydrolysis product ('X') (0.64, solvent system C) could not be matched to any known homologue of dimethoxyglucose in the lit. [12], which included some uncertain data published by Hirst and Jones [13]. Hirst *et al.*'s [14] R_G values for other compounds, e.g. 3-methoxyglucose, 2,3-dimethoxyglucose and 2,3,6-trimethoxyglucose are lower than Rafique and Smith's [15] values using the same solvent system (Table 3). In solvent system C, Hirst's values for glucose and 3-methoxyglucose (0.09 and 0.26, respectively) are also lower than our findings (0.18 and 0.38, respectively). Apparently not knowing the discrepancies in R_G values, Seshadri and Vydeeswaran [6] assumed that the hydrolysis product ('X') could be either 2,6-dimethoxyglucose or 2,4-dimethoxyglucose and, subsequently, ruled out the latter by the periodate test. Since the correct R_G value of our well proven 3,6-dimethoxyglucose (**10**) was 0.61 instead of Hirst *et al.*'s [14] value of 0.51, 'X' can also be a 3,6-homologue. Therefore, Seshadri and Vydeeswaran's [6] formulation of 'X' as a 2,6-homologue was not conclusive and the structure of high mp pariphyllin can be not only $Rha_1 \rightarrow {}_4(Ara_1 \rightarrow {}_3Glu)$-diosgenin but also $Rha_1 \rightarrow {}_4(Ara_1 \rightarrow {}_2Glu)$-diosgenin. Similarly, Singh *et al.* [5] followed the same mistake of overtrusting Hirst *et al.*'s [14] R_G value of 3,6-dimethoxyglucose and then assumed that his low mp polyphyllin D was an isomer, in its glycosidic linkage, of Vydeeswaran's pariphyllin. By the same reason of a revised R_G value of 'X', we predict that polyphyllin D may also be an isomer of $Rha_1 \rightarrow {}_4(Ara_1 \rightarrow {}_2Glu)$-diosgenin.

In order to clarify the inconsistencies in the lit., the Himalayan species of *Paris polyphylla* was collected in 1982 through the herb shops in Nepal. The tubers were extracted, separated and purified in the same way as the other species in this paper. The major products were identified as **1** by TLC with the three solvent systems (A, B and D), mp, IR and ^{13}C NMR. The permethylation of **1** from Nepal (**1**-Nepal) yielded **7**-Nepal which had mp, IR and ^{13}C NMR also completely identical to **7**. After methanolysis, **7**-Nepal yielded **8**-Nepal which was proved to be identical to **8** by GC/MS and further confirmation was obtained by GC/MS of their TMSi-derivatives as listed in Table 2. The mass spectrum of TMSi-**8**-Nepal having ruled out the possibility of its being a 2,6-homologue.

In conclusion, our work shows that the major component of Himalaya species was **1** and the proposed structure of polyphyllin D should be revised. In all likelihood the major saponins from different sources of *P. polyphylla* and from yunnan paiyao are one and the same compound. Apparently, the reported structure of pariphyllin [6] with mp 294–298° is correct.

EXPERIMENTAL

Mps were uncorr. IR spectra were taken in KBr pellets. TLC was performed on silica gel (Camag DF-5) using a 30% H_2SO_4 spray as the visualization reagent. Decending PC was on Whatman No. 1 paper using 10% $AgNO_3$ in NH_4OH for staining. CC was done on silica gel, 230–400 mesh, unless otherwise specified The following solvent systems were used for TLC, CC and PC: solvent A, $CHCl_3$–MeOH–*n*-PrOH–H_2O (70:20:6:3); B, $CHCl_3$–MeOH–H_2O (14:6:1); C, *n*-BuOH–EtOH–H_2O (5:1·4, top layer); D, $CHCl_3$–MeOH–H_2O (60:23:4); E, $CHCl_3$–MeOH (98:2); and F, *n*-hexane–EtOAc (1:1). GC analyses were carried out with FID using He at 20 ml/min, column 2 m × 4 mm packed with 3% SE-30, temp programmed at 110° for 16 min and then increased to 230° at 16°/min for methylated sugars and at 60–230° at 16°/min for TMSi-glucosides. ^1H NMR spectra were recorded at 90 MHz and ^{13}C NMR at 62.8 MHz in δ using TMS as int. standard; $CDCl_3$ was used for permethylated glycosides and $CDCl_3$–MeOH (1:1) for saponins. A VG 70–70F mass spectrometer was used for EI, GC/MS and DCI (NH_3) MS, and a VG ZAB model for FABMS. *Paris polyphylla* var. *chinensis* Hare and *P. polyphylla* Smith (for reference purpose) were purchased from the National Herbal Supplies, Shanghai and confirmed by the Department of Horticulture, South China Agricultural College, Canton, China. Yunnan paiyao was purchased from China Products, Hong Kong.

Extraction and isolation of saponins. *Paris polyphylla* var *chinensis* Hare, sliced tubers (500 g) were extracted with petrol

and the residue extracted with $CHCl_3$–MeOH (1:1) at 40°. The combined $CHCl_3$–MeOH extract (60.1 g) was chromatographed over silica gel, 70–230 mesh, eluted with solvent A. The fractions which contained two spots of the same R_f values on TLC with solvent B were combined for re-chromatography over silica gel, 230–400 mesh with solvent A. Four saponins were obtained after recrystallization from MeOH [R_f, wt (g)]: **1**, 0.55, 3.85; **2**, 0.51, 0.45; **3**, 0.45, 0.06 and **4**, 0.43, 1.9. Saponin (**1**): mp 275–280°; $[\alpha]_D$ −113° (MeOH; *c* 0.53); IR ν_{max}^{KBr} cm^{-1}: 3414 broad, 901 > 920; ^{13}C NMR: δ38.0, 30.2, 78.0, 38.3, 140.9, 122.2, 32.3, 32.2, 50.9, 37.7, 21.5, 39.0, 40.4, 56.6, 32.7, 81.7, 62.9, 16.6, 19.6, 41.6, 14.7, 109.4, 31.9, 29.3, 30.8, 67.5, 17.3 (aglycone C-1–C-27), 101.1, 78.0, 77.2, 78.2, 75.8, 62.4 (glucose moiety C-1–C-6), 101.4, 71.5, 71.9, 73.5, 69.1, 17.7 (rhamnose C-1–C-6), 109.4, 82.0, 77.4, 85.6, 61.5 (arabinose C-1–C-5). FABMS *m/z* (rel. int.): 855 $[M+H]^+$ (19), 723 $[855-Ara+H_2O]^+$ (9), 577 $[723-Rha+H_2O]^+$ (6), 415 $[577-Glu+H_2O]^+$ (75), 397 $[diosgenin-H_2O]^+$ (100). Saponin (**2**): mp 290–294°; $[\alpha]_D$ −114.5° (MeOH; *c* 0.26); FABMS. 869 $[M+H]^+$ (32), 723 (8), 577 (5), 415 (60), 397 (100). Saponin **3**: mp 287–291°; $[\alpha]_D$ −86.1° (MeOH; *c* 0.33); FABMS: 885 $[M+H]^+$ (40), 723 (8), 577 (6), 415 (77), 397 (100). Saponin **4**: mp 217–223°; $[\alpha]_D$ −118.8° (MeOH; *c* 0.59); FABMS. 1015 $[M+H]^+$ (64), 869 (20), 723 (31), 577 (22), 415 (78), 397 (100).

Yunnan paiyao (YNP) (500 g) was extracted, chromatographed and re-crystallized in the same manner as described above to produce 620 mg **1**-YNP, 52 mg **2**-YNP, 20 mg **3**-YNP and 535 mg **4**-YNP, which were found to be identical to **1**–**4**, respectively, in mp, mmp R_f (solvents A and B), IR and MS.

P. polyphylla, grown in the Himalaya region of Nepal, were treated in the same way. Dry tubers (100 g) yielded 260 mg **1**-Nepal, which was found to be identical to **1** and **1**-YNP in mp, mmp, R_f (solvents A, B and D), IR, MS and ^{13}C NMR.

Hydrolysis of saponins. Saponin (**1**) (200 mg) in 7 ml dioxane and 7 ml 2 M H_2SO_4 was hydrolysed at 100° for 4 hr to yield glucose, rhamnose and arabinose in the aq. fraction, and diosgenin in the organic fraction. mp 203–204°; R_f 0.98 (solvent B); $[\alpha]_D$ −127.5° ($CHCl_3$; *c* 0.38); MS *m/z* 414.3126 (calc. for $C_{27}H_{42}O_3$: 414.3134) [16] and ^{13}C NMR [8] identical to the published data. Similarly, **2**–**4** were hydrolysed in the same way to yield glucose, rhamnose and diosgenin.

Partial hydrolysis of saponins. Saponin (**1**) (500 mg) in 10 ml dioxane and 15 ml 0.5 M H_2SO_4 was hydrolysed at 75° for 2 hr. To the resulting mixture, 40 ml *n*-BuOH and 80 ml H_2O were added. The organic layer, after working-up and chromatography on silica gel and elution with solvent A, yielded trillin (45 mg): mp 265–270° from MeOH; R_f 0.75 (solvent B); $[\alpha]_D$ −98.2° (MeOH; *c* 0.39); DCIMS *m/z* (rel. int.): 594 $[M+H+NH_3]^+$ (20), 577 $[M+H]^+$ (28), 415 $[577-Glu+H_2O]^+$ (100), 397 $[415-H_2O]^+$ (55). Also produced from **1** was **5** (125 mg): mp 242–246° from MeOH; R_f 0.6 (solvent B); IR ν_{max}^{KBr} cm^{-1}: 901 > 922, $[\alpha]_D$ −98° (MeOH; *c* 0.27); DCIMS *m/z* (rel. int.): 740 $[M+H+NH_3]^+$ (1), 723 $[M+H]^+$ (11), 577 (2), 415 (35), 397 (100), and unreacted **1**.

Similarly, **1**-YNP and **1**-Nepal were partially hydrolysed under the same conditions to yield trillin and **5**-YNP, and trillin and **5**-Nepal, respectively, with identical mp, R_f (solvents A and B), IR and MS to the partial hydrolysis products from *P. polyphylla* var *chinensis* Hare.

Permethylation of **5** *followed by methanolysis.* A sample of **5** (110 mg) was methylated [17] to yield 84 mg **11**: mp 169–170° from MeOH; $[\alpha]_D$ −97.7° ($CHCl_3$, *c* 0.61); IR: no OH; ^1H NMR δ3.37–3.67 (6*s*, 6 × OMe); DCIMS *m/z* (rel. int.). 824 $[M+H+NH_3]^+$ (18), 807 $[M+H]^+$ ($C_{45}H_{74}O_{12}$) (100). For methanolysis, 70 mg **11** in 1 ml 1 M HCl in MeOH was heated at 100° for 1 hr in a tube previously sealed under N_2. After working-up in the usual manner, the neutral fraction was chromatographed over silica gel and eluted with 100 ml $CHCl_3$–MeOH (99:1) and then 200 ml 2% MeOH in $CHCl_3$. Fraction 1, which showed two TLC spots of R_f 0.76 and 0.55 (solvent E), was analysed by GC/MS and gave two peaks: (i) GC: RR_t 9 min, MS *m/z* (rel. int.): 189 $[M-31]^+$ ($C_{10}H_{20}O_5$) (4), 101 $[CH_3O-CH=CH-CH=OCH_3]^+$ (90), 88 $[CH_3O-CH=CH-OCH_3]^+$ (100), which was assigned for tetramethoxyrhamnose (**12**); (ii) GC: RR_t 139 min, MS: identical to diosgenin. Fraction 2 or **6**, R_f 0.17, (solvent E), produced one GC peak of 19.5 min with MS identical to methyl 3,4,6-methoxyglucoside as listed in Table **1**. Compound **6** was converted into TMSi-**6** according to Pierce's [18] method 2 with MS identical to TMSi-methyl 3,4,6-methoxyglucoside.

Permethylation of **1** *followed by methanolysis.* Saponin (**1**) (500 mg) was permethylated [17] to yield **7** (450 mg): mp 162–163°, $[\alpha]_D$ −113.1° ($CHCl_3$; *c* 0.55); IR ν_{max}^{KBr} cm^{-1}: 902 > 922; ^1H NMR: δ3.37–3.68 (8*s*); DCIMS *m/z* (rel. int.): 984 $[M+H+NH_3]^+$ (30), 967 $[M+H]^+$ ($C_{52}H_{86}O_{16}$) (100). In methanolysis, 100 mg **7** was heated at 100° for 1 hr with 1 ml 1 M HCl in MeOH in a sealed tube under N_2. The product was worked-up and chromatographed in the same way as described for the methanolysis of **11**. Fraction 1 from CC was analysed by GC/MS to produce three peaks (i) GC: RR_t 7.5 min, MS *m/z* (rel. int.): 175 $[M-31]^+$ ($C_9H_{18}O_5$) (3), 161 $[M-45]^+$ (8), 101 (100), 88 (16), 75 $[CH_3O-CH=OCH_3]^+$ (75), 45 $[CH_3O=CH_2]^+$ (80), assigned for tetramethoxyarabinose (**13**). (ii) GC: RR_t 9.0 min, MS: identical to **12**. (iii) GC: RR_t 138 min, MS. identical to diosgenin. In GC/MS, fraction 2 or **8** [R_f 0.06 (solvent E), GC: RR_t 23 min], produced MS similar to both methyl 3,6-methoxyglucoside and methyl 2,6-methoxyglucoside as listed in Table **2**. Compound **8** (1 mg) was converted into TMSi-**8** in the same way as described for TMSi-**6** to produce MS identical to TMSi-methyl-3,6-methoxyglucoside.

Permethylation of **1**-*Nepal followed by methanolysis.* In the same way, 100 mg **1**-Nepal was permethylated to yield 89 mg **7**-Nepal, which was found to be identical to **7** in mp, mmp, R_f (solvents E and F), IR and MS. On methanolysis, **7**-Nepal (10 mg) was converted to **8**-Nepal and then to TMSi-**8**-Nepal, which were found to be identical to the corresponding **8** and TMSi-**8**, respectively in GC/MS as listed in Table 2.

Hydrolysis of **7**. A sample of **7** (10 mg) and 0.1 ml aq. soln of 4 M trifluoroacetic acid in a 5 ml ampoule was sealed under N_2 and heated at 110° for 4 hr. The product, after removal of acid, was analysed by PC in solvent C. Two spots of sugar were found on PC: (i) R_G 0.98 which was assigned for 2,3,5-trimethoxyarabinose (R_G 0.95) and 2,3,4-trimethoxyrhamnose [13]; (ii) R_G 0.61 which was assigned for 3,6-dimethoxyglucose (**10**) since that methyl 3,6-methoxyglucoside was produced from methanolysis of **7**.

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