3. Hasegawa, S., Ou, P., Fong, C. H., Herman, Z., Coggins, W. C., Jr. and Atkin, D. R. (1990) *J. Agric. Food Chem.* **39**, 262.
4. Bennett, R. D. and Hasegawa, S. (1981) *Tetrahedron* **37**, 17.
5. Hasegawa, S., Bennett, R. D. and Herman, Z. (1986) *Phytochemistry* **25**, 1984.
6. Herman, Z., Bennett, R. D., Ou, P., Fong, C. H. and Hasegawa, S. (1987) *Phytochemistry* **26**, 2247.
7. Hasegawa, S., Herman, Z., Ou, P. and Fong, C. H. (1988) *Phytochemistry* **27**, 1349.
8. Ozaki, Y., Miyake, M., Maeda, H., Ifuku, Y., Bennett, R. D., Herman, Z., Fong, C. H. and Hasegawa, S. (1991) *Agric. Biol. Chem.* **55**, 137.
9. Hasegawa, S., Bennett, R. D. and Verdon, C. P. (1980) *J. Agric. Food Chem.* **28**, 922.

Phytochemistry, Vol. 31, No. 3, pp. 1046–1048, 1992
Printed in Great Britain.

0031-9422/92 $5.00+0.00

A BIDESMOSIDIC OLEANOLIC ACID SAPONIN FROM *PANAX PSEUDO-GINSENG**

YOGENDRA N. SHUKLA, RAGHUNATH S. THAKUR and PETER PACHALY†

Central Institute of Medicinal and Aromatic Plants, Lucknow 226016, India; †Pharmaceutical Institute, University of Bonn, Germany

(*Received* 3 *June* 1991)

Key Word Index—*Panax pseudo-ginseng* subsp. *himalaicus* var. *angustifolius*; Araliaceae; rhizomes; triterpenoid saponin pseudo-ginsenoside-RI$_3$.

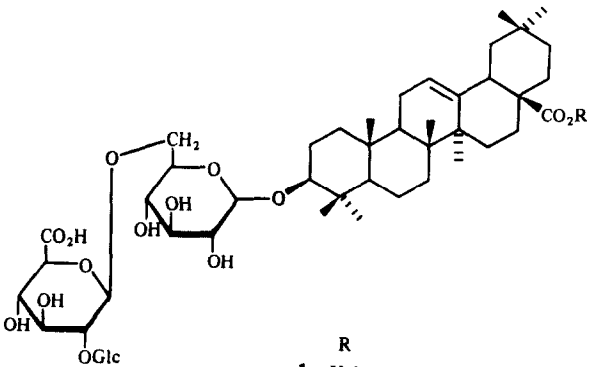
Abstract— A novel triterpenoid saponin, pseudo-ginsenoside-RI$_3$, isolated from the rhizomes of *Panax pseudo-ginseng* subsp. *himalaicus* var. *angustifolius* has been characterized as 3-*O*-*β*-D-glucopyranosyl(1→2)-*β*-D-glucuronopyranosyl (1→6)-*β*-D-glucopyranosyl 28-*O*-*β*-D-xylopyranosyl-olean-12-en-28-oic acid ester by physicochemical methods.

INTRODUCTION

In continuation of our work on the rhizomes and leaves of three varieties of Indian pseudo-ginseng [1], we have recently established that rhizome saponins possess promising adaptogenic and immunostimulant activities which are comparable with Korean ginseng [2]. Further work on the active fraction of *Panax pseudoginseng* subsp. *himalaicus* var. *angustifolius* (Burk.) Li has led to the isolation and characterization of a further new saponin, pseudo-ginsenoside-RI$_3$.

RESULTS AND DISCUSSION

Repeated column chromatography followed by preparative TLC of the saponin mixture obtained from the rhizomes afforded **1**, whose structure was deduced as 3-*O*-*β*-D-glucopyranosyl(1→2)-*β*-D-glucuronopyranosyl-(1→6)-*β*-D-glucopyranosyl 28-*O*-*β*-D-xylopyranosyl-olean-12-en-28-oic acid ester. The IR spectrum of **1** indicated the presence of hyroxy, ester, *gem*-dimethyl and double bond functions. Acid hydrolysis yielded oleanolic acid (**2**), D-glucose, D-xylose and D-glucuronic acid (identified by comparison with authentic samples). Its ^{1}H NMR spectrum exhibited four anomeric signals at



R
1 Xyl
3 H

δ 5.72 (*d*, *J* = 7 Hz), 5.22 (*d*, *J* = 6 Hz), 4.90 (*d*, *J* = 7 Hz) and 5.10 (*d*, *J* = 6.5 Hz) which were consistent with the *β*-configuration for D-xylose, D-glucuronic acid, D-glucose and D-glucose. The ^{13}C NMR spectrum of **1** showed four anomeric signals at *δ* 103.6, 103.9, 103.4 and 94.1 for four sugar residues. The signal at *δ* 94.1 indicated that one of the sugars was linked to C-28 of the aglycone as an ester. This was further confirmed by the alkaline hydrolysis of **1**, which afforded xylose and prosaponin (**3**). Twenty-three carbon signals were seen for the sugar moieties indicating the presence of three hexoses and one pentose, the

*Part 13 in the series 'Studies on Indian ginseng'. For Part 12 see ref. [1] CIMAP Publication No. 49/91.

remaining 30 signals were due to the aglycone. The downfield shift of ~11 ppm for C-3 and ~5 ppm for C-28, and an upfield shift of ~2 ppm for C-2 and ~0.5 ppm for C-17 as compared with **2** confirmed the site of sugar linkage, both at C-3 and C-28 [3].

The chemical shift values of sugar carbons in the ^{13}C NMR spectrum showed the presence of a terminal glucose. The interglycosidic linkage of the sugars was determined from the ^{13}C NMR spectrum of **1** which showed downfield shift values for C-6 of inner glucose. This indicated that C-6 of the glucose residue is linked to C-1 of glucuronic acid and C-2 of glucuronic acid is attached to C-1 of terminal glucose. These observations are consistent with those of related saponins [4]. Close examination of glycosidation [5] as well as acetylation shifts [6] confirmed 2-linked glucuronic acid and 6-linked glucose.

The FAB mass spectrum (positive mode) of **1** showed the $[M+Na]^+$ peak at *m/z* 1111 corresponding to the molecular formula $C_{53}H_{84}O_{23}$. The loss of one xylose was shown by an ion at *m/z* 979 and further fragments at *m/z* 817, 641 and 479 indicated the successive losses of glucose, glucose + glucuronic acid and glucose + glucuronic acid + glucose from the fragment peak at *m/z* 979. This sugar sequence was also consistent to some extent with the results obtained by the partial hydrolysis of compound **1**. The data for acetate **4** are given in Tables 1, 2 and the Experimental. The results obtained above are in full accord with the structure of this saponin as **1**.

Table 1. ^{13}C NMR spectral data of the aglycone moieties of compounds **1–4** (**1–3** in pyridine-d_5, **4** in $CDCl_3$)

C	1	2	3	4
1	38.94	38.90	38.94	38.74
2	26.50	28.20	26.50	26.65
3	88.20	77.70	88.20	88.90
4	39.50	39.40	39.50	39.30
5	55.80	55.80	55.80	55.70
6	18.50	18.80	18.50	18.48
7	33.30	33.30	33.30	33.43
8	39.80	39.80	39.80	39.85
9	48.05	48.10	48.05	48.10
10	37.00	37.20	37.00	37.01
11	23.80	23.80	23.80	23.78
12	122.50	122.51	122.50	122.60
13	144.80	144.80	144.80	144.65
14	42.00	42.05	42.00	42.12
15	28.30	28.30	28.30	28.25
16	23.80	23.80	23.80	23.85
17	47.28	46.80	46.80	46.65
18	42.00	42.00	42.00	42.10
19	47.00	46.80	47.00	47.18
20	30.90	31.00	31.00	31.00
21	34.30	34.30	34.30	34.30
22	33.30	33.30	33.30	33.10
23	28.70	28.70	28.70	28.65
24	16.64	16.50	16.60	16.10
25	15.45	15.50	15.45	15.25
26	17.70	17.50	17.60	17.45
27	26.20	26.20	26.20	26.18
28	175.12	180.20	180.30	175.00
29	33.30	33.30	33.25	33.20
30	23.80	23.80	23.78	23.75

Table 2. ^{13}C NMR spectral data of the sugar moieties of compounds **1**, **3** and **4**

	1	3	4
3-*O*-Sugar moieties			
Glc (inner)			
1	103.55	103.55	102.50
2	75.25	75.25	74.60
3	78.00	78.00	76.15
4	72.15	72.15	73.50
5	76.35	76.35	75.00
6	69.87	69.87	69.14
Glucuronic acid			
1	103.86	103.86	100.10
2	81.20	81.20	80.05
3	77.80	77.80	76.60
4	73.20	73.20	75.18
5	78.30	78.30	76.22
6	172.50	172.50	172.00
Glc (terminal)			
1	103.40	103.40	99.00
2	76.30	76.30	74.30
3	78.10	78.10	81.00
4	71.50	71.50	72.80
5	78.10	78.10	77.10
6	60.68	60.68	60.37
28-*O*-Sugar moieties Xyl			
1	94.10		91.00
2	76.70		72.80
3	77.61		75.68
4	71.10		73.10
5	60.85		60.78
MeCO			168–70
MeCO			19–21

The saponins in which glucuronic acid is directly attached to the aglycone are commonly found but those saponins such as **1** in which glucuronic acid is not directly linked to aglycone are rare. Some of them are isolated earlier from *Anemone raddeana* [7], *Akebia quinata* [8] and *Buplerum falcatum* [9].

EXPERIMENTAL

Mps: uncorr. ^1H NMR spectra: 300 MHz with TMS as int. standard, ^{13}C NMR spectra: 75 MHz. EIMS was obtained with direct inlet system at 70 eV while the positive ion mode FAB-MS was obtained using glycerol as matrix. IR spectra were recorded as KBr disc. CC was carried out on silica gel (B.D.H., 60–120 mesh) and RP-8 material, and TLC and prep. TLC on silica gel 60 precoated plates, F-254 (Merck). The spots on TLC were visualized by spraying with 10% H_2SO_4 followed by heating at 110°. PC was performed on Whatmann No. 1 paper using the descending mode and developed with aniline hydrogen phthalate. The following solvent systems were employed: (A) $CHCl_3$–MeOH–H_2O (13:7:2, lower phase), (B) $CHCl_3$–MeOH–H_2O (6:4:1), (C) MeOH–H_2O (13:7), (D) *n*-BuOH–HOAc–H_2O (4:1:5), (E) *n*-BuOH–HOAc–H_2O (6:3:1). Plant material was collected from Singalila Range, Darjeeling, West Bengal and a voucher specimen is deposited in our Botany Department.

Extraction and isolation of saponin. Dried and powdered rhizomes (268 g) of *P. pseudo-ginseng* subsp. *himalaicus* var.

angustifolius were extracted with EtOH (50%, 9 × 600 ml). The extract was freed of the solvent *in vacuo* and the residue redissolved in EtOH (50%, 300 ml). It was then extracted successively with *n*-hexane (6 × 200 ml, 3 g) and *n*-BuOH (7 × 200 ml, 68 g). The *n*-BuOH fraction (10 g) was chromatographed over silica gel (350 g) eluting with solvent A. Frs (100 ml each) were collected and each was monitored by TLC. Compound **1** was obtained from eluates 33–45 which was further purified by passing through RP-8 in solvent C and prep. TLC in solvent B.

Compound **1**. Amorphous, 150 mg, mp 290–295° (dec.) (MeOH), $[\alpha]_D$ +6.5° (pyridine). IR ν_{max}^{KBr} cm^{-1}: 3420, 2950, 1730, 1700, 1450, 1385, 1365, 1165, 1070, 820. ^1H NMR (DMSO-d_6): δ0.82, 0.84, 0.86, 0.88, 0.90, 1.02, 1.08 (3H, each *s*, 7 × *tert*. Me), 5.38, (1H, *m*, H-12), 5.72 (1H, *d*, *J* = 7 Hz, Xyl H-1), 5.22 (1H, *d*, *J* = 6 Hz, GlcUA H-1), 5.10 (1H, *d*, *J* = 6.5 Hz, Glc H-1), 4.90 (1H, *d*, *J* = 7 Hz, Glc H-1), 3.12–4.50 (Xyl, GlcUA and 2 × Glc), 2 78 (1H, *dd*, *J* = 18 and 4 Hz, H-18).

Acidic hydrolysis of compound **1**. Saponin **1** (40 mg) was refluxed with methanolic HCl (5%, 25 ml) for 4 hr to furnish the aglycone (oleanolic acid) as needles, mp 297–298°, $[\alpha]_D$ +85° ($CHCl_3$). MS *m/z* 456 $[M]^+$, identified by authentic sample comparison (co-TLC, MS, ^1H and ^{13}C NMR). The neutralized (Ag_2CO_3) and concd aq. hydrolysate indicated the presence of D-glucuronic acid, D-xylose and D-glucose (co-PC and co-TLC with authentic sugars, solvents, D, E).

Alkaline hydrolysis of compound **1**. Saponin **1** (40 mg) was hydrolysed with methanolic KOH (5%, 20 ml) for 2 hr. After work-up prosaponin (**3**) was obtained. Analysis of the aq. layer by TLC and PC confirmed the presence of xylose.

Prosaponin **3**. ^1H NMR (DMSO-d_6): δ0.82, 0.84, 0.86, 0.88, 0.90, 1.02, 1.08 (3H, each *s*, 7 × *tert* Me), 5.38 (1H, *m*, H-12), 2.80 (1H, *dd*, *J* = 18 and 4 Hz, H-18), 5.22 (1H, *d*, *J* = 6 Hz, GlcUA H-1), 5.10 (1H, *d*, *J* = 6 Hz, Glc H-1), 4.90 (1H, *d*, *J* = 7 Hz, Glc H-1), 3.10–4.52 (GlcUA, 2 × Glc).

Partial acid hydrolysis of compound **1**. Saponin **1** (10 mg) was hydrolysed with methanolic HCl (5%, 2 ml) for 10 min. After usual work-up the aq. hydrolysate showed the presence of glucose and xylose (co-PC with authentic samples).

Acetylation of compound **1**. Saponin **1** (45 mg) was treated with Ac_2O–pyridine (each 1 ml) overnight at room temp. The usual work-up afforded an acetate **4**, $[\alpha]_D$ +25.2° ($CHCl_3$). IR ν_{max}^{KBr} cm^{-1}: 2960, 2850, 1700, 1725–1750 (br), 1450, 1380, 1360, 1250. ^1H NMR ($CDCl_3$): δ0.82, 0.84, 0.86, 0.88, 0.90, 1.02, 1.08 (3H, each *s*, 7 × *tert* Me), 1.98–2.18 (12 × OAc), 5.72 (1H, *d*, *J* = 7 Hz, Xyl H-1), 5.22 (1H, *d*, *J* = 7 Hz, GlcUA H-1), 5.10 (1H, *d*, *J* = 6 Hz, Glc H-1), 4.90 (1H, *d*, *J* = 7 Hz, Glc H-1), 5.34 (1H, *m*, H-12), 2.80 (1H, *dd*, *J* = 18 and 4 Hz, H-18), 3.12–4.80 (GlcUA, Xyl and Glc).

Acknowledgements—The authors are grateful to Dr J. Peter (Institute for Physiological Chemistry, Bonn) for FAB-MS. Part of this work was carried out at Pharmaceutical Institute, Bonn, Germany under DAAD-CSIR exchange programme and also supported by the International Foundation for Science, Stockholm, Sweden, Project No. 535.

REFERENCES

1. Shukla, Y. N. and Thakur, R. S. (1991) *Indian Drugs* **28**, 249.
2. Dua, P. R., Shanker, G., Srimal, R. C., Saxena, K. C., Saxena, R. P., Puri, A., Dhawan, B. N., Shukla, Y. N., Thakur, R. S. and Husain, A. (1989) *Indian J. Exp. Biol.* **27**, 631.
3. Tanaka, O. and Kasai, R. (1984) in *Progress in the Chemistry of Organic Natural Products* Vol. 46 (Herz, W., Grisewach, H., Kirby, G. W. and Tamm, Ch., eds), p. 26. Springer, Vienna.
4. Ahmad, V. U., Perveen, S. and Bano, S. (1990) *Phytochemistry* **29**, 3287.
5. Tori, K., Seo, S., Yoshimura, Y, Nakamura, M., Tomita, Y. and Ishii, H. (1976) *Tetrahedron Letters* 4167.
6. Terui, Y., Tori, K. and Tsuji, N. (1976) *Tetradehron Letters* 621.
7. Wu, F. and Zhu, Z. (1983) *Lanzhou Daxue Xuebao Ziron Kexuban* **19**, 188; (1984) *Chem. Abs.* **100**, 135, 780.
8. Higuchi, R. and Kawasaki, T. (1972) *Chem. Pharm. Bull. Jpn* **20**, 2143.
9. Vecherko, L. P., Zinkevich, E. P. and Kogan, L. M. (1973) *Khim. Prir. Soedin.* **9**, 561.