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AN ACETYLATED SAPONIN FROM *PANAX PSEUDO-GINSENG* SUBSP. *HIMALAICUS* VAR. *ANGUSTIFOLIUS**

YOGENDRA N. SHUKLA and RAGHUNATH S. THAKUR

Central Institute of Medicinal and Aromatic Plants, Lucknow, 226016, India

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Key Word Index—*Panax pseudo-ginseng* subsp. *himalaicus var. angustifolius*; Araliaceae; rhizomes; pseudo-ginsenoside-RI$_1$ and RP$_1$.

Abstract—The structure of a new acetylated saponin isolated from the rhizomes of *Panax pseudo-ginseng* subsp. *himalaicus* var. *angustifolius* has been elucidated as 3-*O*-[*β*-D-4-acetylxylopyranosyl (1→2)-*β*-D-glucuronopyranosyl]-oleanolic acid by physico-chemical methods.

INTRODUCTION

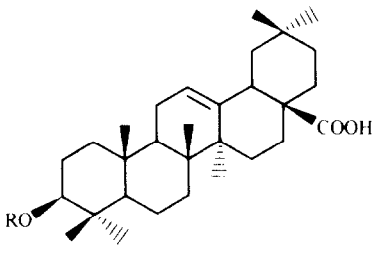
Recently we have reported 13 known saponins [1, 2] and several other constituents [1, 3, 4] from the rhizomes of *Panax pseudo-ginseng* Wall. subsp. *himalaicus* Hara var. *angustifolius* (Burk.) Li (Araliaceae). Since ginseng has been attributed with a wide variety of medicinal properties, our continued interest in this plant has led to the isolation and structure elucidation of a new acetylated saponin named pseudo-ginsenoside-RI$_1$.

RESULTS AND DISCUSSION

Repeated column chromatography and preparative TLC of the complex saponin mixture obtained from the rhizomes of this plant afforded compound **1**, which was characterized as 3-*O*-[*β*-D-4-acetylxylopyranosyl(1→2)-*β*-D-glucuronopyranosyl]-oleanolic acid. IR spectrum of this compound showed the presence of –OH, –OAc, –COOH, *gem* dimethyl and double bond functions. Acid hydrolysis furnished oleanolic acid (**2**), D-xylose and D-glucuronic acid. Alkaline hydrolysis of **1** yielded **3** which lacked acetate bands and was identified as pseudo-ginsenoside-RP$_1$ [2, 5] by spectral data and its acidic hydrolysis products, **2**, D-xylose and D-glucuronic acid. Formation of **3** from **1** clearly demonstrated that both have similar sequence and linkages of sugars. It was also in full accord with their 100 MHz ^{13}C NMR data (Table 1).

400 MHz ^{1}H NMR spectrum of **1** displayed two one proton doublets at δ 5.76 (J = 7 Hz) and δ 4.90 (J = 6 Hz) which were assigned to *β*-anomeric protons of glucuronic acid and xylose, respectively. The downfield shift of ~11 ppm for C-3 in the ^{13}C NMR of **1** and an upfield shift of ~2 ppm for C-2 as compared with **2** confirmed the site of sugar linkage at C-3 [6].

Presence of an acetyl group in **1** was shown by IR bands at 1730 and 1270 cm^{-1}, ^{1}H NMR signal at δ 2.02 and ^{13}C NMR signals at δ 170.8 and 20.90. The EIMS of **1** showed fragment peaks at *m/z* 456 [oleanolic acid]$^+$, 351 [(glcUA-Xyl) Ac]$^+$, 175 [(terminal Xyl) Ac]$^+$, 133 [175 −42]$^+$ and 115 [175 − AcOH]$^+$. This evidence indicated that **1** must be a monoacetate of **3** and the acetyl group of **1** was located at the terminal xylosyl unit of 3-*O*-*β*-diglycosyl moiety of **3**. In partially acetylated glycosides the location of acyl linkage is determined by ^{13}C NMR spectroscopy. On acylation, a carbinyl carbon signal is somewhat deshielded and the carbon signals of *β*-positions are displaced upfield, while other carbon signals of the alcohol moiety remain almost unaffected [7 9]. In the ^{13}C NMR, on going from **3** to **1**, carbon signals due to C-3 and C-5 of xylose moiety were displaced upfield by 2.5 and 2.3 ppm, respectively whereas the C-4 signal was deshielded by 2.5 ppm. The other signals remained almost unshifted. These data confirmed that acetyl group in **1** was present at the 4-OH group of terminal xylosyl unit.



1 R = *β*-D-Glc UA^2—*β*-D-Xyl^4—Ac
2 R = H
3 R = *β*-D-Glc UA^2—*β*-D-Xyl

* Part 5 in the series 'Studies on Indian ginseng'. For part 4 see ref. [2]. CIMAP Communication No. 727.

Table 1. ^{13}C-NMR data of compounds **1–3** in δ ppm (pyridine)

	Aglycone moiety			Sugar moiety					
C	1	2	3	1		3			
1	38.86	38.90	38.70	3-GlcUA 1	105.80	3-GlcUA 1	105.20		
2	26.65	28.20	26.70	2	83.50	2	83.50		
3	89.29	78.00	89.30	3	77.80	3	77.80		
4	39.62	39.40	39.60	4	73.20	4	73.20		
5	56.03	55.80	55.90	5	78.10	5	78.10		
6	18.65	18.80	18.50	6	172.40	6	172.20		
7	33.38	33.30	33.30	Xyl 1	107.12	Xyl 1	106.90		
8	39.94	39.80	39.70	2	76.60	2	76.60		
9	48.18	48.10	48.00	3	74.80	3	77.30		
10	37.15	37.40	37.00	4	73.60	4	71.10		
11	23.91	23.80	23.80	5	65.20	5	67.50		
12	122.67	122.50	122.50	MeCO	170.80				
13	144.95	144.80	144.80	MeCO	20.90				
14	42.34	42.00	42.20						
15	28.47	28.30	28.40						
16	23.91	23.80	23.80						
17	46.82	46.70	46.70						
18	42.17	42.00	42.00						
19	46.68	46.70	46.50						
20	31.06	31.00	31.00						
21	34.44	34.30	34.30						
22	33.38	33.30	33.30						
23	28.38	28.70	27.90						
24	16.40	16.50	16.40						
25	15.56	15.50	15.50						
26	17.53	17.50	17.40						
27	26.31	26.20	26.20						
28	180.24	180.20	180.20						
29	33.38	33.30	33.30						
30	23.91	23.80	23.80						

GlcUA = β-D-Glucuronopyranosyl; Xyl = β-D-xylopyranosyl.

EXPERIMENTAL

Mps: uncorr. ^{13}C NMR spectra were recorded at 100 MHz and ^{1}H NMR spectra at 400 MHz with TMS as int. standard. EIMS were obtained with a direct inlet system at 70 eV and IR spectra were determined as KBr discs. CC was performed on Silica gel (BDH, 60–120 mesh) and 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. PC was carried out on Whatman No. 1 paper using the descending mode and developed with aniline hydrogen phthalate. The following chromatographic solvent systems were used: (A) $CHCl_3$–MeOH–H_2O (13:7:2, lower phase), (B) $CHCl_3$–MeOH–H_2O (6:4:1, homogeneous), (C) $CHCl_3$–MeOH (9:1), (D) *n*-BuOH–HOAc–H_2O (4:1:5), (E) *n*-BuOH–HOAc–H_2O (6:3:1), (F) $CHCl_3$–MeOH (1:3). Plant material was collected from the Singalila range of Darjeeling, West Bengal and identified in our Botany Department where a voucher specimen No. 1927 has been preserved.

Extraction and isolation of compounds. Dried and powdered rhizomes (268 g) of *P. pseudo-ginseng* subsp. *himalaicus* var. *angustifolius* were extracted with EtOH (50%, 9 × 600 ml). The solvent was removed *in vacuo* and the residue was dissolved in EtOH (50%, 300 ml). It was then successively extracted with *n*-hexane (6 × 200 ml, 3 g) and *n*-BuOH (7 × 200 ml, 68 g). Part of the *n*-BuOH fraction (40 g) was chromatographed over silica gel (1.2 kg), eluting with increasingly polar mixtures of $CHCl_3$ and MeOH. Fractions (250 ml each) were collected and each were monitored by TLC. Compound **1** was obtained from the solvent F eluates 541–564 (2 g) whereas the other eluates yielded saponins reported earlier. These eluates afforded homogeneous **1** on repeated CC (solvent B) and prep. TLC (solvent A).

Compound 1. Colourless needles, 86 mg, mp 222–226° (*d*) (MeOH), $[\alpha]_D$ +19.05° (1, pyridine). IR ν_{max} cm^{-1}: 3400, 2940, 2860, 1730, 1695, 1610, 1460, 1385, 1360, 1270, 1210, 1165, 1070, 1040, 820. ^{1}H NMR (C_5D_5N): δ0.84, 0.85, 0.86, 0.90, 0.94, 0.96, 1.06 (3H, each *s*, 7 × *tert.* Me), 5.34 (1H, *m*, H-12), 2.02 (3H, *s*, OAc), 5.76 (1H, *d*, *J* = 7 Hz, glcUA H-1), 4.90 (1H, *d*, *J* = 6 Hz, xyl H-1), 3.62–4.56 (9H, glcUA and Xyl). (Found: C, 63.85; H, 8.53% $C_{43}H_{66}O_{14}$ requires: C, 64.01; H, 8.18%).

Alkaline hydrolysis of 1. Saponin **1** (50 mg) was refluxed with methanolic KOH (5%, 10 ml) for 2 hr to provide compound **3**, mp 230–232°, $[\alpha]_D$ +4° (MeOH). It was identified by comparison with authentic **3** (co-TLC, mp, IR and ^{13}C NMR).

Acidic hydrolysis of 1 *and* 3. Compounds **1** (25 mg) and **3** (35 mg) were refluxed separately with methanolic HCl (5%, 10 ml) for 4 hr to afford the aglycone (oleanolic acid), colourless needles, mp 298–299°, $[\alpha]_D$ +85° ($CHCl_3$); MS *m/z*: 456 $[M]^+$; identified by comparison with authentic material (co-TLC, mmp, ^{1}H NMR, IR, and ^{13}C NMR) [10]. The neutralized (Ag_2CO_3)

and concd aq. hydrolysate from each showed the presence of D-glucuronic acid and D-xylose (co-PC and co-TLC with authentic sugars, solvents D.E).

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REFERENCES

1. Shukla, Y. N. and Thakur, R. S. (1986) *Phytochemistry* **25**, 2201.
2. Shukla, Y. N. and Thakur, R. S. (1988) *Planta Med.* (in press).
3. Shukla, Y. N. and Thakur, R. S. (1985) *Phytochemistry* **24**, 1091.
4. Shukla, Y. N. and Thakur, R. S. (1987) *Indian J. Pharm. Sci.* **49**, 140.
5. Tanaka, O., Morita, T., Kasai, R., Kinouchi, J., Sanada, S., Ida, Y. and Shoji, J. (1985) *Chem. Pharm. Bull.* **33**, 2323.
6. Tanaka, O. and Kasai, R. (1984) in *Progress in the Chemistry of Organic Natural Products* (Herz, W., Grisewach, H., Kirby, G. W. and Tamm, Ch., eds) Vol. 46, p. 26. Springer, Vienna.
7. Yamasaki, K., Kasai, R., Masaki, Y., Yokihara, M., Tanaka, O., Oshio, H., Takagi, S., Yamaki, M., Masuda, K., Nonaka, G., Tsuboi, M. and Nishioka, I. (1977) *Tetrahedron Letters* 1231.
8. Asen, A. and Horowitz, R. M. (1977) *Phytochemistry* **16**, 147.
9. Chari, V. M., Jordan, M., Wagner, H. and Thies, P. M. (1977) *Phytochemistry* **16**, 1110.
10. Nie, R. L., Morita, T., Kasai, R., Jhou, J., Wu, C. Y. and Tanaka, O. (1984) *Planta Med.* **50**, 322.

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A NOVEL STILBENE FROM THE WOOD OF *CHLOROPHORA EXCELSA*

LARS P. CHRISTENSEN, JØRGEN LAM* and TORBEN SIGSGAARD†

Department of Organic Chemistry, University of Aarhus, 8000 Aarhus C, Denmark;† Institute of Hygiene Medicine, University of Aarhus, 8000 Aarhus C, Denmark

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Key Word Index—*Chlorophora excelsa*; *Moraceae*; chlorophorin; 4-geranyl-3,5,4′-trihydroxy-*trans*-stilbene.

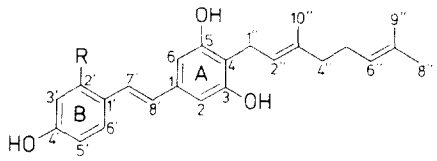
Abstract—A novel stilbene, has been isolated from the diethyl ether extract of the wood of *Chlorophora excelsa* and its structure established as 4-geranyl-3.5,4′-trihydroxy-*trans*-stilbene through spectral studies.

INTRODUCTION

The West African timber *Chlorophora excelsa* (Bentham and Hooker), is generally known under the trade names Iroko and Kambala. It is resistant to fungus and insect attack and is known to cause a cell mediated type of allergy (allergic contact dermatitis) [1]. The allergenic principle of this timber has been identified as chlorophorin [2, 3]. In the present paper we report the isolation and structure elucidation of a novel stilbene from *Chlorophora excelsa*. Its sensitizing potency is not known, but is under investigation.

RESULTS AND DISCUSSION

From the diethyl ether extract of the wood of *Chlorophora excelsa* two stilbenes were isolated by flash chromatography and prep. TLC. These included a new stilbene (**1**) and the known chlorophorin (**2**) [4–7]. Moreover a third compound, possibly a stilbene, is present in a mixture with **1** showing a molecular ion peak at *m/z* 378. This compound could not be separated from **1** in a pure state, which made the structure determination impossible. The main compound was **2** with the molecular formula $C_{24}H_{28}O_4$. The obtained spectroscopic data (see Experimental and Table 1) confirmed the already estab-



1 R = H
2 R = OH

*Author to whom correspondence should be addressed.