Phytochemistry, Vol. 28, No. 7, pp. 1855–1858, 1989.
Printed in Great Britain.

0031-9422/89 $3.00 + 0.00

DAMMARANE AND OLEANANE SAPONINS FROM CALLUS TISSUE OF *PANAX JAPONICUS*

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(*Received in revised form* 7 *December* 1988)

Key Word Index—*Panax japonicus*; Araliaceae; tissue culture; callus; triterpenes; dammarane saponin; oleanane saponin; ginsenosides; chikusetsusaponins.

Abstract—From tissue callus of *Panax japonicus* grown in South Kyushu, two ginseng dammarane saponins, ginsenosides Rg_1 and Re, and an oleanane saponin (desglucosylchikusetsusaponin-IV) together with chikusetsusaponin-IV were isolated.

INTRODUCTION

Panax japonicus is a perennial herb indigenous to Japan and China. Panacis Japonici Rhizoma (Chikusetsu-ninjin, in Japanese), the dried rhizome of this plant, has been utilized for several hundred years in Japan, sometimes as a substitute for the well-known herbal medicine, *P. ginseng*. However, these two species contain different types of saponin, although some are common to both. In general, *P. ginseng* contains many dammarane saponins, namely ginsenosides-Rg_1, -Re, -Rc, and -Rb_1, while *P. japonicus* contains a large amount of oleanane saponins, such as chikusetsusaponins-IV and -V, and a small amount of dammarane saponins, chikusetsusaponins-Ia and III [1].

Recently, Satsuma-ninjin, a chemotype of *P. japonicus* of limited distribution in South Kyushu, Japan was reported. The composition of saponins in the rhizome of this plant was remarkably different from that of other *P. japonicus* specimens collected from other places in Japan [2]. It contains many dammarane saponins, including ginsenosides-Re, -Rg_1, Rb_1, -Rc, etc. which are known as major saponins of *P. ginseng*, and had not been detected previously from *P. japonicus*. Also, it contains oleanane saponins, chikusetsusaponin-IV and -V which are common constituents of *P. japonicus*. However, it lacks two dammarane-saponins, chikusetsusaponins-Ia and III which are characteristic constituents of *P. japonicus*. From a callus induced from the rhizome of normal *P. japonicus* collected in Hiroshima prefecture, we have already isolated six oleanane saponins, and identified two as chikusetsusaponins-IV and IVa, which were constituents of the original plant [3].

For the purpose of producing medicinally interesting ginseng saponins from inexpensive sources, callus was induced from *P. japonicus* collected in South Kyushu (Satsuma-ninjin), and constituents of the callus investigated. Another purpose of this work was to determine whether the anomalous saponin constitution of Satsuma-ninjin is intrinsic. In this paper, we report the isolation and identification of two dammarane saponins, ginsenosides Rg_1 and Re along with two oleanane saponins, chikusetsusaponin-IV and desglucosylchikusetsusaponin-IV from the callus of Satsuma-ninjin.

RESULTS AND DISCUSSION

Callus tissue cultures were established from the flower bud of *P. japonicus* collected in South Kyushu and they were cultured in the dark on Murashige and Skoog's medium containing 2,4-D. Different culture conditions with IBA and kinetin instead of 2,4-D, which was used in a previous study on *P. japonicus* of Hiroshima origin, was also carried out for comparison. Since the TLC pattern obtained from both culture procedures did not show any difference, the former medium was used throughout this study.

The TLC patterns of the saponin fraction of the methanol extract of the callus and the original plant (rhizome) were compared with those of Hiroshima origin. Differences according to origin were clearly observed in the callus as well as in the rhizome samples [2]. To confirm the difference, constituents were isolated. From the methanol extract, four saponins **1**–**4** were obtained by silica gel and reversed phase chromatography.

The TLC profiles of **1**–**3** were identical with known constituents of Satsuma-ninjin, namely ginsenoside Rg_1, ginsenoside Re and chikusetsusaponin-IV, respectively, and the ^{13}C NMR spectra of **1**–**3** were in excellent agreement with published data (Tables 1 and 2) [1, 4].

Compound **4** was obtained as a white powder, $[\alpha]_D^{25} = -22.3°$ (pyridine; *c* 0.27). Its ^{13}C NMR spectrum showed a strong resemblance to that of **3** lacking, however, six signals corresponding to an ester glucosyl moiety. Aglycone (oleanolic acid) and 4-arabinosyl glucuronyl carbon signals were almost superimposable with that of **3** except the C-28 carboxyl and the vicinity (C-22, -19, -13 and -12). The chemical shifts of these exceptional signal were in

Part 4 in the series 'Studies on the tissue culture of *Panax japonicus*'. For Part 3 see ref. [3].

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Table 1. ^{13}CNMR data of compounds **1**, **2** and reference compounds in C_5D_5N (δ values, 100 MHz, TMS int std)

		Protopanaxatriol*	Ginsenoside-Rg$_1$*	**1**	Ginsenoside-Re*	**2**
Aglycone moiety						
1		39.2	39.5	39.5[a]	39.4	39.2
2		28.0	27.6	27.9	27.4	27.0
3		78.3	78.6	78.7	78.7	78.5
4		40.2	40.1	40.3	39.8	39.7
5		61.7	61.3	61.4	60.7	60.8
6		67.6	77.8	78.0	74.6	74.9
7		47.4	44.9	45.2	45.7	45.6
8		41.0	41.0	41.2	41.0	41.0
9		50.1	49.9	50.0	49.4	49.5
10		39.3	39.5	39.7[a]	39.4	39.5
11		31.9	30.8[b]	31.0[b]	30.6	30.3
12		70.9	70.3	70.3	70.3	70.6
13		48.1	48.9	49.2	48.8	48.6
14		51.6	51.3	51.4	51.3	51.4
15		31.3	30.6[b]	30.7[b]	30.6	30.8
16		26.8	26.4	26.7	26.5	26.5
17		54.6	51.6	51.7	51.8	52.0
18		17.5[a]	17.4[a]	17.5[c]	17.4[a]	17.3[a]
19		17.4[a]	17.4[a]	17.5[c]	17.4[a]	17.3[a]
20		72.9	83.3	83.4	83.2	83.8
21		26.9	22.3	22.4	22.4	22.5
22		35.7	35.9	36.2	35.8	35.8
23		22.9	23.2	23.3	23.3	23.4
24		126.2	125.8	126.0	125.7	125.6
25		130.6	130.9	130.9	130.9	131.5
26		25.8	25.7	25.7	25.7	25.7
27		17.7[a]	17.7[a]	17.8[c]	17.7[a]	17.8[a]
28		31.9	31.6	31.7	32.0	31.8
29		16.4[a]	16.2[a]	16.3[c]	17.1[a]	17.1[a]
30		17.0[a]	17.0[a]	17.2[c]	17.1[a]	17.1[a]
Sugar moiety						
6-Glc	1		105.7	105.9	101.6	101.5
	2		75.3	75.5	79.1	78.9[b]
	3		80.0[c]	80.1[d]	78.0[b]	77.7[c]
	4		71.6[d]	72.0	72.1	72.1[d]
	5		79.3[c]	79.5[d]	78.0[b]	77.6[c]
	6		62.9	63.2[c]	62.9	62.7
Rha	1				101.6	101.4
	2				72.1	71.7[d]
	3				72.1	71.9[d]
	4				73.8	73.6
	5				69.3	69.5
	6				18.6	18.3
20-Glc	1		98.1	98.2	98.1	97.9
	2		74.9	75.1	75.0	74.9
	3		78.8[c]	79.2[d]	79.1	78.6[b]
	4		71.3[d]	71.7	71.7	70.8
	5		77.8[c]	78.1[d]	78.5[b]	78.1[c]
	6		62.9	62.9[c]	62.7	62.1

*Cited from ref. [4].

[a–c] Assignments in the vertical column may be interchanged.

Glc: β-glucopyranosyl, Rha: α-L-arabinofuranosyl.

good agreement with the aglycone, oleanolic acid (Table 2). It was noted that the chemical shift of C-28 in **4** was slightly shifted downfield from that of the aglycone due to salt formation (**4** was not deionized by ion exchange resin). The same phenomenon was observed at C-6 of the glucuronic acid moiety. Thus, the 28 carboxyl group of **4** was free. Consequently, the structure of **4** was confirmed to be 3-(4-α-L-arabinofuranosyl)-β-D-glucuronosyl olean-

Table 2. ^{13}C NMR data of compounds **3**, **4** and reference compounds in C_5D_5N, (δ values, 100 MHz, TMS int std)

		Oleanolic acid	IV*	3	4	4 (ref. [6])
Aglycone moiety						
1		38.9	38.6	38.6	38.4	n.r.†
2		28.2	26.5	26.2	26.1	
3		78.0	89.1	89.3	89.7	
4		39.4	39.5	39.3	39.2	
5		55.8	55.7	55.7	55.7	
6		18.8	18.3	18.4	18.3	
7		33.3	33.1	33.0	33.2	
8		39.8	39.9	39.8	39.5	
9		48.1	48.0	47.9	47.8	
10		37.4	36.9	36.8	36.7	
11		23.8	23.7	23.7	23.6	
12		122.5	122.8	122.9	122.4	
13		144.8	144.1	144.0	144.7	
14		42.0	42.1	42.1	42.0	
15		28.3	28.2	28.1	28.1	
16		23.8	23.7	23.6	23.6	
17		46.7	47.0	47.0	46.9	
18		42.0	41.7	41.7	42.0	
19		46.7	46.2	46.2	46.5	
20		31.0	30.8	30.6	30.7	
21		34.3	33.9	33.9	34.1	
22		33.3	32.6	32.4	33.0	
23		28.7	28.2	28.1	28.0	
24		16.5	16.9	16.8	16.7	
25		15.5	15.5	15.4	15.2	
26		17.5	17.5	17.4	17.3	
27		26.2	26.1	26.0	26.1	
28		180.2	176.3	176.7	181.5	
29		33.3	33.1	33.0	33.2	
30		23.8	23.7	23.3	23.6	
Sugar moiety						
GlcA	1		107.0	106.4	106.0	106.7
	2		75.2	75.1	74.8	75.5
	3		76.1[a]	76.1	75.9	76.3‡
	4		78.8	78.8	79.4	78.6§
	5		76.6[a]	78.1[a]	78.0	77.3
	6		172.4	175.4	175.4	174.3
Ara	1		108.6	108.9	109.0	108.6
	2		82.4	82.3	82.3	82.3§
	3		78.0	78.2[a]	77.8	77.8‡
	4		87.8	86.2	85.6	87.4
	5		62.6	62.6	62.5	62.8
Glc	1		95.7	95.5		
(28)	2		74.1	73.7		
	3		79.2[b]	79.0[b]		
	4		71.1	70.9		
	5		78.8[b]	78.8[b]		
	6		62.2	62.0		

*Chikusetsusaponin-IV, cited from ref. [4].

† n.r.: not reported.

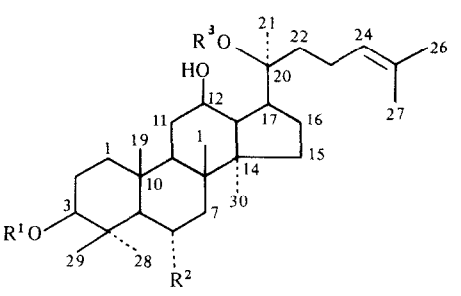
[a,b] Assignments in the same vertical column may be interchanged.

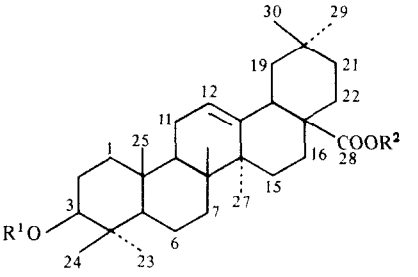
GlcA: β-glucuronic acid, Ara: α-L-arabinofuranosyl, Glc: β-glucopyranosyl.

‡§ Assignment different from that reported in ref. [6].

olic acid. This compound is a prosapogenin of chikusetsusaponin-IV, and has been obtained from Himalayan ginseng, *P. pseudo-ginseng* subsp. *pseudo-ginseng* in an impure state without characterization by ^{13}C NMR data [5]. Isolation of **4** from *Cussonia spicata* of the same family has also been described [6], but the reported ^{13}C NMR data are only for sugar carbons.

The results we obtained are sufficient to prove that the





Dammarane saponins				Oleanane saponins		
	R^1	R^2	R^3		R^1	R^2
C – 1a:	– Glc(6→1)Xyl	– H	– H	C – 1b:	– GlcA(4→1)Ara, Ara(6→1)Glc	– H
C – III:	– Glc(2→1)Glc, Glc(6→1)Xyl	– H	– H	C – IVa:	–GlcA	– Glc
G – Rb_1:	– Glc(2→1)Glc	– H	– Glc(6→1)Glc	C – IV: (3)	– GlcA(4→1)Ara	– Glc
G – Rc:	– Glc(2→1)Glc	– H	– Glc(6→1)Ara	Pro-C – IV (4)	– GlcA(4→1)Ara	– H
G – Rg_1: (1)	– H	– O – Glc	– Glc			
G – Re: (2)	– H	– O – Glc(2→1)Rha	– Glc	C – V:	– GlcA(2→1)Glc	– Glc

C –: chikusetsusaponin G –: ginsenoside– Pro –: prosapogenin–

Glc: β – D – glucopyranosyl
Xyl: β – D – xylopyranosyl
Rha: α – L – rhamnopyranosyl
Ara: α – L – arabinofuranosyl
GlcA: β – D – glucuronopyranosyl

regional strain of *P. japonicus* is rather intrinsic and that Satsuma-ninjin itself and its callus are expected to provide a new source of valuable ginseng saponins.

EXPERIMENTAL

Plant material. Callus tissue cultures were established from flower buds of *P. japonicus* C. A. Meyer collected in Takakuma Experimental Forestry at Kagoshima University (South Kyushu) and cultured in the dark on Murashige and Skoog's medium containing 2,4-D (1 ppm). Different culture conditions (IBA, 10^{-4} M and kinetin, 10^{-6} M instead of 2,4-D), the same as those used in a previous study on *P. japonicus* from Hiroshima were also tried for comparison.

Extraction and isolation. Callus tissues were lyophilized (105 g) and extracted with hot MeOH. The material (41 g) was chromatographed on a column of a highly porous polymer, Diaion HP-20 eluting with 50% MeOH. MeOH and $CHCl_3$, successively. The MeOH eluent (3.7 g) was chromatographed on a silica gel column using $CHCl_3$–MeOH–H_2O mixts to give 9 frs. From the first fr. (128 mg), compound **1** (10 mg) was isolated by reversed phase (RP_{18}, MeOH–H_2O, 3:2) and silica gel CC($CHCl_3$– MeOH–H_2O, 15:6:1). From the fourth fr, compounds **2** (8 mg) and **4** (13 mg) were isolated by reversed phase (RP_{18}, MeOH–H_2O, 3:2 and Diposil C_{18}, dil MeOH)CC. From the eighth fr., **3** (21 mg) was obtained after RP_{18}CC (60% MeOH).

Acknowledgements—We are indebted to Prof. T. Nohara and Dr S. Yahara (Kumamoto University) for ^{13}C NMR measurements.

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