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Isolation of Toxic Saponins from the Roots of *Patrinia scabiosaefolia*

Won Sick Woo, Jae Sue Choi and Kuk Hyun Shin
Natural Products Research Institute, Seoul National University, Seoul 110, Korea

Abstract—During the biological screening of Chinese drugs, it was found that alcoholic extract of the roots of *Patrinia scabiosaefolia* (Valerianaceae) caused a significant prolongation of hexobarbital-induced sleeping time and elevation of serum transaminase activities accompanied by severe histopathological changes in the hepatic tissues in mice on three day pretreatments. The systematic fractionation of the methanol extract monitoring by bioassay led to isolation of toxic saponins such as 3-O-α-L-arabinopyranosyl hederagenin 28-O-β-D-glucopyranosyl (1→6)-β-D-glucopyranoside and its 2′-acetate and 3-O-β-D-glucopyranosyl (1→3)-α-L-rhamnopyranosyl (1→2)-α-L-arabinopyranosyl oleanolic acid and its 28-O-β-D-glucopyranosyl (1→6)-β-D-glucopyranoside.

Keywords—*Patrinia scabiosaefolia* · Valerianaceae · saponins · hepatotoxins

The number of drugs used in treatment or prevention of diseases in man is uncountable. They can be divided broadly into two catagories: first, drugs used in western medicine and second, drugs used in traditional oriental medicine. The drugs used by western theraphy have been mainly synthetic compounds or pure isolates from natural products. They were discovered effective to some specific diseases but some of them were later discovered to produce side effects.

On the contrary, drugs used in oriental medicine have been mostly natural herb preparations and people believe them to be less toxic and almost without side effects. Nevertheless most of them have hardly recognized therapeutic utility in western medicine. But even so some of them have been often reported to be effective in healing problems that have proved recalcitrant to standard western methods. Therefore, Chinese herb theraphy has received increasing popularity in spite of disadvantage that its mechanism of action is difficult to be explained scientifically and its toxicic effects have not been well established. However, as a matter of fact, every herb drug is not considered to be safe. There is a possibility that some herb drugs may have a toxic constituent. Hence they should be subjected to the re-evaluation for efficacy as well as safety.

In the course of biological screening of Chinese drugs in our laboratory it was found that the alcohol extract from the roots of *Patrinia scabiosaefolia* (Valerianaceae) did not affect hexobarbital induced sleeping time in mice on a single pretreatment. However, on repeated pretreatment, significant prolongation of hexobarbital induced sleeping time was observed.[1] This unexpected and interesting results suggested the liver damage caused by the plant material rather than its pharmacological effect. In order to support this suggestion serum transaminase activities which are known to increase extremely in acute liver poisoning were checked in animals given

the plant extract daily for 3 days. As expected, the plant extract increased activities of GOT and GPT significantly accompanied by histopathological changes in the liver tissue.[2]

In order to separate toxic constituents the methanol extract was fractionated through solvent partition into four fractions such as hexane soluble fraction, chloroform soluble fraction, butanol soluble fraction and water soluble fraction and each fraction was tested for activities of hexobarbital induced sleeping time prolongation and transaminase activity elevation. Butanol soluble fraction and chloroform soluble fraction prolonged hexobarbital induced sleeping time; butanol fraction showed more strong activity than chloroform fraction.[3] All fractions elevated serum transaminase activities and among them the butanol fraction was by far the strongest one.[3]

Therefore the butanol fraction was subjected to column chromatography monitoring with animal tests, to yield four toxic compounds. Compounds **1**, mp 224~226°, $[\alpha]_D^{25}$ −0.01°, gave a positive reaction in the Lieberman Burchard test and Molisch test. Its IR spectrum showed the presence of ester group (1725 and 1250cm^{-1}) and glycoside bond (1000~1100 cm^{-1}). Judging from its NMR spectrum it was found that it contained one acetyl group (PMR; δ1.98, CMR; δ20.8 and 169.1) and at least three moles of sugars (CMR; δ103.0, 102.4 and 94.0). Compound **1** gave compound **2**, mp 218~220°, $[\alpha]_D^{25}$ +16°, by mild alkaline hydrolysis (2.5% K_2CO_3). Compound **1** and **2** gave hederagenin as the aglycone and L-arabinose and D-glucose as the sugar moieties on acid hydrolysis. FAB mass spectrum indicated the molecular weight of compound **2** was 928. This fact together with an appearance of three anomeric carbon signals in its CMR spectrum (δ104.6, 103.0 and 94.1) clearly indicated that compound **2** consisted of one mole of hederagenin, one mole of L-arabinose and two moles of D-glucose. The appearance of ester absorption bands in IR (1735 cm^{-1}) and carboxyl carbon signal at rather highfield in CMR (δ175.2) strongly indicated that one of sugar moieties was linked to 28-carboxyl function of the genin. Alkaline hydrolysis of compound **2** gave prosapogenin A, mp 228~231°, $[\alpha]_D^{25}$ +67.2°, which was hydrolyzed by acid treatment into hederagenin and L-arabinose, and enzymatic hydrolysis broke the molecule to liberate prosapogenin B, mp 204~206°, $[\alpha]_D^{20}$ +30°, which was hydrolyzed by acid treatment into hederagenin, L-arabinose and D-glucose. These facts indicated that a disaccharide chain composed of two glucose units was bonded to the 28 carboxyl group of prosapogenin A by an ester linkage. Methanolysis of permethylated compound **2**, mp 96~98°, prepared according to Hakomori's method[4] afforded 23-O-methylhederagenin, methyl 2,3,4-tri-O-methyl-L-arabinopyranoside, methyl-2,3,4-tri-O-methyl-D-glucopyranoside and methy 2,3,4,6-tetra-O-methyl-D-glucopyranoside, indicating that L-arabinose was linked to 3-position of the genin rather than 23-position and the disaccharide bonded to the carboxyl group should be gentiobiose. PMR of this compound showed three doublets at δ4.14 (J=6.5Hz), 4.20 (J=8Hz) and 5.35 (J=8Hz) assignable to anomeric protons of L-arabinose, terminal D-glucose and inner D-glucose, respectively. The relatively large coupling constants indicated the β-configuration for both glucoside linkages and α-configuration for arabinoside linkage. Therefore compound **2** is a hederagenin glycoside consisting of 1 mole of arabinose attached to 3-position and 1 mole of gentiobiose to 28-position.

Table I shows CMR spectral data of hederagenin and its glycosides obtained. All the chemical shifts of sapogenin carbons are not shown in this Table because those for each compound are almost the same except those of C-3, 23 and

Table I. ^{13}C-NMR chemical shift of compound 1, 2, and prosapogenins.

Carbon	Hed	I	II	A	B
Genin					
C-3	70.7	80.0	80.1	80.0	80.0
C-23	65.1	62.9	62.9	62.9	62.9
C-28	178.2	175.2	175.2	178.6	175.1
Ara					
C-1		102.4	104.6	104.8	104.5
C-2		73.1	71.1	71.2	71.1
C-3		70.5	72.7	72.9	72.7
C-4		68.1	67.4	67.6	67.4
C-5		65.5	64.9	65.1	64.8
COMe		20.8			
COMe		169.1			
Glu					
C-1		94.0	94.1		94.1
C-2		72.3	72.3		72.4
C-3		76.7	76.7		76.7
C-4		69.4	69.4		69.7
C-5		76.7	76.7		77.6
C-6		68.1	68.0		60.8
t-Glu					
C-1		103.0	103.0		
C-2		73.4	73.5		
C-3		76.7	76.7		
C-4		70.0	70.1		
C-5		76.6	76.6		
C-6		61.0	61.0		

28. The carbon 3 signal of hederagenin appeared at 70.7 ppm. However those of the glycosides were found to be shifted to 80 ppm indicating the site of glycosidation. The carbon 23 signals were displaced upfield owing to the glycosidation effect. Chemical shifts of arabinose moiety of compound 2 were superimposable with those of the prosapogenins. This indicated that glucose was not linked to any hydroxyl group of arabinose.

C-28 signals of the compound 1 and 2 and prosapogenin B appeared at high field compared those of hederagenin and prosapogenin A, indicating 28-carboxyl group of those compounds was esterified.

Carbon 6 signal of D-glucose of prosapogenin B appeared at 60.8 ppm. However the corresponding carbon signal of compound 2 was shifted downfield by+7.2 ppm and C-5 signal was shifted upfield by −0.9 ppm, while other resonances appeared almost unshifted. This indicated that the second D-glucose must be located at 6-position of inner D-glucose.

Chemical shift values of anomeric carbon of each sugar clearly indicated α-configuration for arabinoside linkage and β-configuration for both glucoside linkages. Moreover consideration of molecular optical rotation by the application of Klyne's rule supported the above mentioned con figurations of all sugar linkages.Observed values of molecular optical rotations of the compounds are in agreement with the calculated values for the proposed structures (Table II).

The attachment point of the acetyl group in compound 1 was determined by the CMR spectral analysis. In comparison between spectral data of compound 1 and 2, all the signals due to the sapogenin and D-glucose moieties of both compounds appeared at almost the same positions. With regard to the arabinose carbon region, on going from compound 2 to compound 1, the

Table II. Molecular optical rotation values of saponins and prosapogenins

Compound	Observed $[M]_D$	Calculated*
Compound 2	+146.88°	+321.17°
Prosapogenin A	+405.89°	+453.17°
Prosapogenin B	+229.8°	+387.17°
Hederagenin	+424.8°	
Compoend 3	+22.66°	+119.57°
Compound 4	− 0.15°	− 12.43°
Prosapogenin C	+55.05°	+185.57°
Prosapogenin D	+294°	+295.57°
Oleanolic acid	+267.2°	

Me-α-L-Ara+28.37° (β-anomer+402.62°), Me-α-L-Rha−110° (β-anomer+168°), Me-β-D-Glu−66° (α-anomer+304°)

* Other possible combinations of anomeric methyl glucosides have been considered for the calculation.

signal for C-2 was displaced dowfield by 2.0 ppm while both signals due to C-1 and C-3 were shielded by −2.2 ppm. Such changes in the chemical shifts can only be explained if the hydroxyl group at 2-position of arabinose moiety is acetylated. Therefore the structure of compound **1** was elucidated as 2′-O-acetylcompound **2**. Compound **3**, mp 258∼260°, $[\alpha]_D^{25}$+2.53°, and **4**, mp 218∼220°, $[\alpha]_D^{25}$−0.012° also gave a positive reaction in the Liebermann-Burchard and Molish tests. On acid hydrolysis compound **3** gave oleanolic acid, L-arabinose, D-glucose and L-rhamnose. Its molecular weight was 896 and three anomeric carbon signals were found in its CMR (δ104.6, 103.9 and 100.0). The absorption peak due to a free carboxyl group appeared at 1705cm^{-1}. These spectral data indicated that compound **3** was an oleanolic acid glycoside consisting of a trisaccharide composed of 1 mole each of L-arabinose L-rhamnose and D-glucose, attached to 3-position of the genin.

Compound **4** was also hydrolyzed by acid treatment into oleanolic acid, L-arabinose, D-glucose and L-rhamnose. However its molecular weight was 1220 and five anomeric carbon signals were found in its CMR (δ104.1, 103.3, 102.9, 99.8 and 94.1). This indicated that compound **4** consisted of one mole each of L-arabinose and L-rhamnose and three moles of D-glucose as sugar moieties. Its IR spectrum showed an absorption peak at 1730cm^{-1} due to ester group and on alkaline hydrolysis compouund **4** gave compound **3**. Therefore compound **4** was suggested to be an ester glycoside of compound **3** with an additional disaccharide of two moles of glucose which was linked to 28-carboxyl group. Partial hydrolysis of compound **3** yielded prosapogenins C, mp 242∼244°, $[\alpha]_D^{25}$+7.5°, and D mp 256∼258°, $[\alpha]_D^{23}$+50°. Prosapogenin C was hydrolyzed by strong acid treatment into oleanolic acid, L-arabinose and L-rhamnose, while prosapogenin D, into oleanolic acid and L-arabinose only. Therefore, in compound **3**, the trisaccharide was linked to the genin in order of L-arabinose, L-rhamnose and D-glucose.

Methanolysis of permethylated compound **3**,

Table III. ^{13}C-NMR Chemical shift of compound **3**, **4**, and prosapogenins.

Carbon	Ole	3	4	C	D
Genin					
C-3	78.8	87.8	87.8	87.7	87.8
C-28	178.3	178.5	175.1	178.3	178.3
Ara					
C-1		103.9	103.3	103.4	105.5
C-2		74.4	74.8	74.7	71.0
C-3		72.5	71.8	71.9	72.7
C-4		67.7	67.0	67.0	67.4
C-5		64.2	63.4	63.2	64.6
Rha					
C-1		100.0	99.8	99.9	
C-2		69.5	69.3	70.3	
C-3		81.5	81.0	70.3	
C-4		70.8	70.7	71.9	
C-5		68.1	68.1	68.3	
C-6		17.8	17.8	17.6	
Glu					
C-1		104.6	104.1		
C-2		74.0	73.7		
C-3		76.7	76.6		
C-4		70.7	70.1		
C-5		76.4	76.4		
C-6		61.1	61.1		
28-Glu					
C-1			94.1		
C-2			72.3		
C-3			76.6		
C-4			69.6		
C-5			76.4		
C-6			68.1		
t-Glu					
C-1			102.9		
C-2			73.4		
C-3			76.4		
C-4			70.2		
C-5			76.4		
C-6			61.1		

mp 220~222°C, gave oleanolic acid methlester, methyl 3, 4-di-O-methyl-L-arabinopyranoside, methyl 2, 4-di-O-methyl-L-rhamnopyranoside and methyl 2, 3, 4, 6-tetra-O-methyl-L-glucopyranoside. Therefore the mode of linkage between arabinose and rhamnose was determined to be 1-2 and that between rhamnose and glucose was 1-3. Three anomeric protons of this compound resonated at δ4.45 (d, J=5Hz), 4.47 (d, J=7Hz) and 5.05 (bs) and from values of the their coupling constants it is clear that configurations of linkages for arabinoside and rhamnoside are α and that for glucoside is β. Therefore structure of compound 3 was established as 3-O-β-D-glucopyranosyl (1→3)-α-L-rhamnopyranosyl (1→2)-α-L-arabinopyranosyl oleanolic acid.

CMR chemical shifts of compound 3 and 4 and the prosapogenins obtained from compound 3 are shown in Table III. As compared with the chemical shifts of arabinose carbons of prosapogenin D, C-2′ signal of prosapogenin C was shifted downfield. In comparison between chemical shifts of rhamnose carbons in compound 3 and prosapogenin C, C-3″ signal of compound 3 was shifted downfield. This supported that C-2 of arabinose and C-3 of rhamnose were the site of glycosidation. In comparison of spectral data of compound 4 with those of compound 3, chemical shifts for the trisaccharide moiety of compound 4 were superimposable with those of the compound 3. However, C-28 signal of compound 4 was shielded due to esterification with a disaccharide. A set of newly appeared signals in the sugar carbon region of the spectrum of compound 4 was completely identical with those for gentiobiose carbons in compound 1 and 2 (Table I). Therefore compound 4 was identified as 28-gentiobioside of compound 3.

Configurations of all sugar linkages were further confirmed by application of Klyne's rule. Observed values of molecular optical rotations of the compounds are in excellent agreement with the calculated values for the assigned structures (Table II). In conclusion toxic saponins in *Patriniae radix* were determined as 3-α-L-arabinopyranosyl hederagenin 28-β-D-glucopyranosyl (1→6)-β-D-glucopyranoside and its 2′-acetate and 3-β-D-glucopyranosyl (1→3)-α-L-rhamnopyranosyl (1→2)-α-L-arabinopyranosyl oleanolic acid and its 28-β-D-glucopyranosyl (1→6)-β-D-glucopyranoside.

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