Korean J. Ginseng Sci
Vol. 7, No. 2 (1983)

Biosynthesis of Saponins in *Panax ginseng* C. A. Meyer

1. Probable sites of the Biosynthesis of ginseng saponin from acetate

Chung-No Joo, Hahn-Sik Kwak, Hui-Bong Lee*, Chong Hwa Lee
Department of Biochemistry, College of Science, Yonsei University, Seoul 120, Korea Ginseng and Tobacco Research Institute
(Received October 6, 1983)

인삼사포닌의 생합성에 관한 연구

1. acetate로 부터 인삼사포닌 생합성의 가능부위

주충노 · 곽한식 · 이휘봉 · 이종화
연세대학교 이과대학 생화학과, 한국인삼연구소
(1983년 10월 6일 접수)

Abstract

1. To know the site of saponin synthesis in this plant, 4-years old *Panax ginseng* C.A. Meyer was administered with 1, 2-^{14}C-acetate (Na salt, 10 uCi/plant) by stem injection and was continued to grow for 3 weeks and the distribution of the radioactivity in leaf, stem and root part was identified. The percentage of radioactivity recovered was about 3.99%.
2. The sliced roots or leaf discs (2g) were bathed in the reaction mixture containing sugar, ATP, NADPH, and the distribution of the radioactivity of the fractions (sugar, saponin, sapogenin) ws identified.
3. It seemed that major synthesized saponins in roots and leaves are diol and triol-type, respectively. Although both types of saponins are synthesized in roots, the main saponins seemed to be diol saponins and a significant portion of triol saponins are supplied from leaves through stem.

Introduction

Saponins exist in plants usually in the form of glycosides and the removal of its attached sugars on hydrolysis yields sapogenins. It is now well established that panax ginseng contained dammarane type glycosides and a little oleanolic-glycosides. Furthermore, it was known that the distribution of dammarane type triterpens, such as ginseng panaxadiol and panaxatriol, appears restricted to the genera *panax* in the Araliaceae family.

During the past twenty years, extensive biochemical and pharmacological studies of ginseng saponin have been carried out by many workers, however, there still remained a number of fundamental problems to be answered to classify the actions of ginseng saponins.

One of the most urgent requirements to solve the above problems was to provide the radioactive saponins. Woo and Han [1,2] have succeeded to introduce ^{14}C-isopropyl fragment to the side chain of panax saponin *A*, one of the ginseng saponins. The also established a synthetic procedure of labelling tritium

in polycyclic skeleton of the saponin. Recently, New England Nuclear have developed the synthetic method of preparing radioactive saponins.[3]

For the purpose of preparing radioactive saponins from 1,2-^{14}C-acetate using tissues of panax ginseng as enzyme source, we have carried out experiment to know the site or sites of saponin synthesis in this plant.

Materials and Methods

Four year old panax ginseng was administered with 1,2-^{14}C-acetate (Na-salt, 10 uCi/plant) by stem injection and the plant was grown for three weeks in this laboratory. The plant was then divided into root, stem and leaf parts.

They were homogenized using water followed by centrifugation, and the insoluble precipitates were washed with water three times. The combined water extract was then mixed with equal volume of methanol, and then heated and left to stand in refrigerator. After centrifugation to remove the insoluble fraction, the supernatant was diluted with 3 volumes of chloroform and centrifuged to remove the insoluble fraction. The supernatant (methanol-chloroform phase) was concentrated under reduced pressure and dissolved in 90% methanol. This alcohol solution was extracted again with petroleum ether to remove fat soluble substances such as sapogenins. The remaining methanol solution was concentrated under reduced pressure and dried in vacuo. The extraction procedure was summarized in Fig. 1.

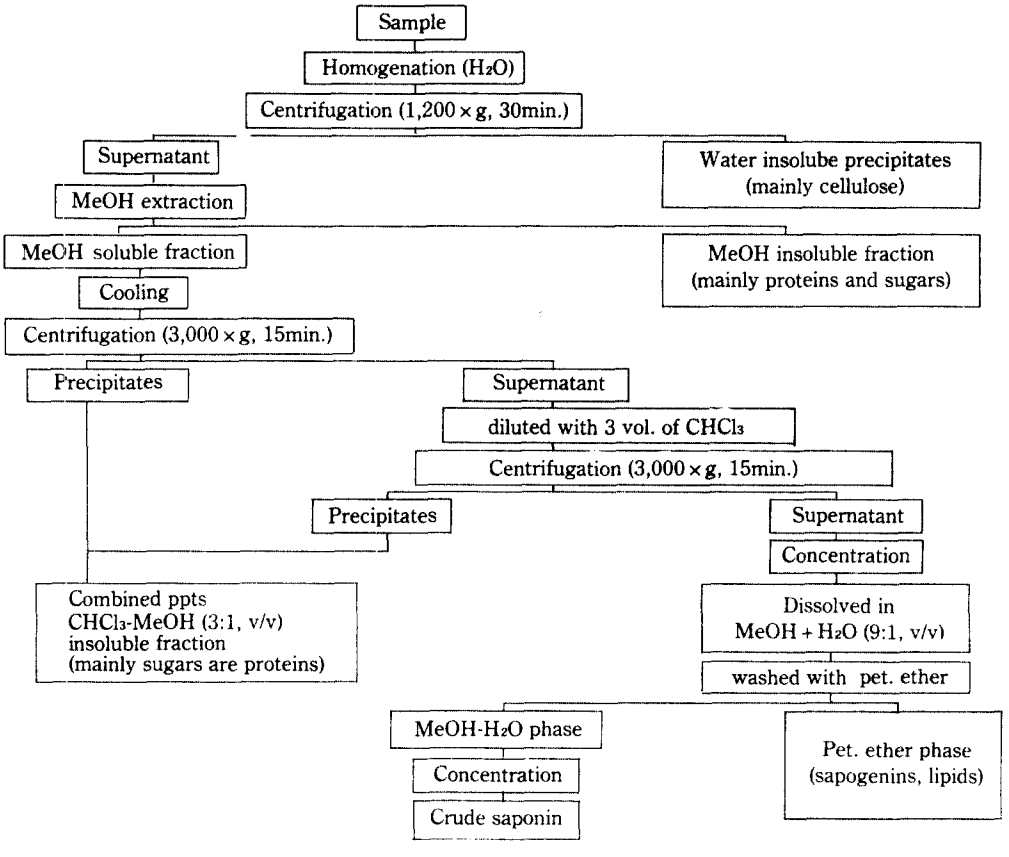

Fig. 1. Summarized scheme of the fractionation of water extract from root, stem, and leaf of *Panax ginseng* C.A. Meyer.

^{14}C-labelled saponins from 1,2-^{14}C-acetate was prepared using either sliced roots or leaf discs of panax ginseng as enzyme sources according to Joo *et al*[4] as shown in Fig. 2.

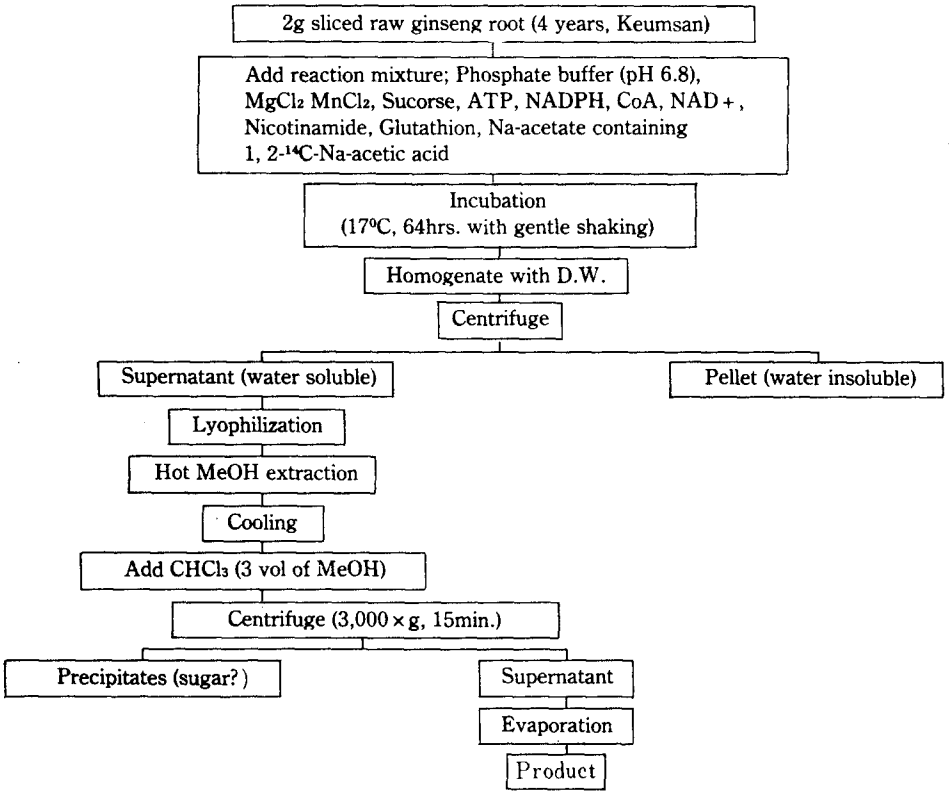

Fig. 2. The preparation of radioactive ginseng saponin.

Silica gel thin layer chromatography was carried out using chloroform: methanol: water (65:40:9, v/v/v) as a developing solvent. Autoradiograph was made by pressing medical X-ray safety film on the thin layer chromatogram for seven days.

The radioactivity was assayed using arkard Tri-Carb 300 liquid scientillation spectrometer. The liquid scientillation cocktails were prepared either by dissolving 10g of PPO, 0.25g of POPOP and 100g of naphthalene in 1000ml of dioxane or by mixing 5g of PPO, 600ml of methoxy ethanol and 1000ml of toluene.

NAD+, Coenzyme A, NADPH were the products of Sigma Chem. Co. Sucrose, precoated silica gel TLC plate, nicotinamide, glutathion were purchased from Merck Co. PPO and POPOP were obtained from Fisher Scientific Co. and 1,2-^{14}C-acetate (Na-salt) was purchased from New England Nuclear.

Results and Discussion

Radioactivity recovered from the whole plant which was administered through stem injection of 1,2-^{14}C-acetate (10 uCi) prior to 3 week growing in this laboratory was found 3.99% suggesting that the acetates are actively metabolized in this plant.

The radioactivity distribution showed that most of the activity was observed in stem part (68%), but a significant amount of the radioactivity was also in roots (29%) but relatively small portion of the radioactivity was recovered from leaves as shown in Table 1.

Table 1. Distribution of radioactivity in root, stem and leaf part of *Panax ginseng* C.A. Meyer.

	Radioactivity (DPM)						
Fraction / Major components	Water insoluble precipitates	MeOH insoluble fraction	$CHCl_3$-MeOH (3:1) insoluble fraction	Pet. ether soluble fraction	Crude saponin fraction	Total	Relative %
Part	Cellulose	Proteins and sugars	Proteins and sugars	Sapogenins and lipids	Saponins		
Root	4,946	67,177	39,999	108,570	33,356	254,048	29.0
(relative %)	(1.95)	(26.44)	(15.74)	(42.74)	(13.13)	(100)	
Stem	113,312		27,310	358,150	97,745	596,517	68.0
(relative %)	(19.00)	trace	(4.58)	(60.04)	(16.39)	(100)	
Leaf	513	4,000	2,789	2,549	16,901	26.752	3.0
(relative %)	(1.92)	(14.95)	(10.43)	(9.53)	(63.18)	(100)	
Whole plant	118,771	71,177	70,098	469,269	148,002	877,317	100
(relative %)	(13.5)	(8.1)	(8.0)	(53.5)	(16.9)	(100)	

* Which was grown for three weeks in laboratory (25°C) after stem injection of 1,2-^{14}C-Na-acetate (10 uCi). The % of radioactivity recovered from the whole plant was 3.99%.

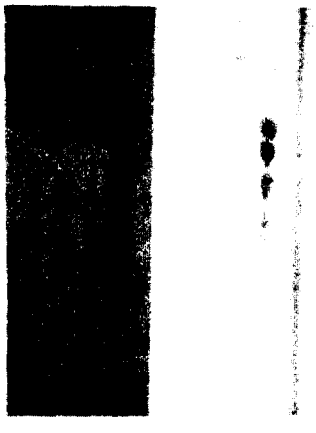

Fig. 3. Chromatogram (A) and its autoradiogram (B) of the water extracts from root (2), stem (3), and leaf (4) and ^{14}C-saponin preparation (1). TLC (pre-coated TLC plate, silica gel 60F-254, 5×20cm) was carried out using $CHCl_3$: MeOH: H_2O (65:40:9, v/v/v) as a developing solvent.

It was noticeable that more than half of the total radioactivity was detected in petroleum ether soluble fraction which contained sapogenins and other lipids. Relatively high proportion of the radioactivity was found in saponin fraction and water insoluble fraction which seemed to be mainly cellulose. It is easily expected that the administered acetate was rapidly metabolized and turned over to sugars.

Table 2 showed that the radioactivity distribution in sapogenin, saponin and sugar fractions separated by thin layer chromatography of water extract from ginseng root. As much as 68.7% of the radioactivity was detected saponin fraction and 31.3% was in sapogenin fraction, but the radioactivity detected in sugar fraction was relatively small suggesting that saponin might be synthesized in roots with the supply of sugars, NADPH and ATP from leaf where these were known to be synthesized.

Autoradiograph of chromatogram of the root extract showed that a strong radioactivity was seen in diol-saponin fraction and less but appreciable amounts of radioactivities were seen in triol-saponin fraction as well as sapogenin fraction but little radioactivity was seen in sugar fraction as shown in Fig. 3.

Both leaf and stem extracts showed that the radioactivities were seen in sugar fraction, triol-saponin fraction and a little in sapogenin fraction but not in diol-saponin fraction suggesting that mainly triol-saponins might be synthesized in leaf. Very high radioactivities in triol-saponin fraction, sapogenin fraction and sugar fraction of the stem (Table 2) suggested that these substances synthesized at leaf might be transported through stem to other part such as roots of this plant.

Table 2. Radioactivity distribution in sugar, saponin and sapogenin fraction of silica gel thin layer chromatograms of water extracts of root, stem, and leaf parts or panax ginseng*

Part \ Fraction	Sugar fraction	Saponin		Sapogenin	Total
		Diol-saponin	Triol-saponin		
Root	trace	4,979	1,936	3,150	10,065
(relative %)		(49.5)	(19.2)	(31.3)	(100)
Stem	3,795	2,892	4,043	13,221	23,951
(relative %)	(15.8)	(12.1)	(16.9)	(55.2)	(100)
Leaf	124	269	383	401	1,177
(relative %)	(10.5)	(22.9)	(32.5)	(34.1)	(100).

* Which was grown for three weeks after the stem injection of 1,2-^{14}C-Na-acetate.

As shown in Fig. 3, the roots contained both diol and triol saponins, but it is uncertain whether the triol saponins were synehesized *in situ* or supplied from the leaf through stem. It was, therefore, carried out to synthesize ^{14}C-saponins from 1,2-^{14}C-acetate using root slices as enzyme source to know whether the root could synthesize both diol-and triol-saponins with the supply of NADPH, ATP and sugars.

When 2g of root slices of panax ginseng were incubated at 37°C for 64 hrs. in the reaction mixture (3.3ml) containing 9.1×10^{-2}M phosphate buffer (pH 6.8), 7.3×10^{-4}M NAD+, 2.3×10^{-2}M nicotinamide, 3.6×10^{-3}M $MgCl_2$, 3.6×10^{-3}M $MnCl_2$, 1.1×10^{-1}M sucrose, 4.5×10^{-3}M ATP, 2.7×10^{-4}M NADPH, 4.5×10^{-4}M CoA, 9.1×10^{-3}M glutathion, 1.1×10^{-2}M Na-acetate and 1,2-^{14}C-Na-acetate (300 uCi), 11.2% of the radioactivity were recovered, of which most of radioactivity was in the saponin fraction as shown in Table 3.

Autoradiogram (Fig. 3) of the chromatogram of the above preparation showed that both diol-and triol-saponins could be synthesized in roots.

Table 3. Radioactivity recovered from the saponin fraction prepared from 1,2-^{14}C-acetate (300 uCi) by incubating the root slices (2g) of Panax ginseng C.A. Meyer*

Fraction	Insoluble ppts.	Sapogenin	Saponin	Radioactivity recovered
Radioactivity (cpm)	23,075,938	51,753	50,716,358	73,844,049 (% recovered = 11.2)
Relative %	32.0	0.1	67.9	100

* at 37°C, for 64 hours in the reaction mixture (3.3ml) containing (9.1×10^{-2}M phosphate butter (pH 6.8), 3.6×10^{-3}M $MgCl_2$, 3.6×10^{-4}M $MnCl_2$, 1.1×10^{-1}M sucrose, 4.5×10^{-3}M ATP, 2.7×10^{-4}M NADPH, 4.5×10^{-4}M CoA, 7.3×10^{-4}M NAD+, 2.3×10^{-2}M nicotinamide, 9.1×10^{-3} glutathion, 1.1×10^{-2}M Na-acetate). After incubation the mixture was extracted and fractionated by preparative chromatography.

It was also attempted to prepare saponins from ^{14}C-acetate (5000 cmp) using leaf discs as enzyme source to know the leaf could synthesize saponins *in situ*.

When 2g of leaf discs were incubated in the same reaction mixture described above when root slices were used as enzyme source, % of the radioactivity recovered was as high as 75% and most of the radioactivity were detected in sugar and triol saponin fractions. This suggested that leaf contained a synthesizing system for both diol and triol saponins.

Table 4. Radioactivity recovered from the saponin preparation using 1,2-^{14}C-acetate (5,000 cpm) by incubating the leaf discs (2g) of panax ginseng*

Fraction	Pet. ether Solubles (sapogenin)	Sugar fraction (Sugars)	Saponin fraction		Radioactivity recovered	Total radioactivity
			Diol saponin	Triol saponin		
Radioactivity (cpm)	367	1,012	982	1,402	3,763	5,000

* at 37°C for 64 hours in the reaction mixture (3.3ml) containing 9.1×10^{-2}M phosphate butter (pH 6.8), 3.6×10^{-3}M $MgCl_2$, 3.6×10^{-3}M $MnCl_2$, 1.1×10^{-1}M sucrose, 4.5×10^{-3}M ATP, 2.7×10^{-4}M NADPH, 4.5×10^{-4}M CoA, 7.3×10^{-4}M NAD+, 2.3×10^{-2}M nicotinamide, 9.1×10^{-3}M glutathione, $1.1\times$ *10* $\pm M$ *Na-acetate.*

From the above results, it was tentatively concluded that both diol-and triol saponins might be synthesized in roots with the supply of sugars, NADP and ATP from leaf through stem, but the former saponins seemed to be synthesized predominantly. Leaf also could synthesize both saponins but triol saponins are mainly synthesized and transported to other parts of this plant through stem.

It seemed that although both the diol-saponin and triol-saponin were synthesized in the roots, but the main synthesizing saponin was diol-saponin and a significant amount of triol-saponin might be supplied from leaves through stem.

요 약

1. 인삼사포닌의 식물체내에서의 합성 부위를 알고자 1,2-^{14}C-Na-acetate를 줄기에 주입하여 3주후 잎, 줄기, 뿌리에서의 방사능 분포를 확인하였다. 이때의 방사능 회수율은 약 3.99%이었다.

2. 뿌리와 잎을 각각 효소원으로 하여 당, ATP, NADPH등을 포함한 반응물에 담가 반응시킨 후 각 분획에서의 방사능 분포를 확인하였다. 뿌리의 경우 방사능 회수율은 약 7.68%이었다.

3. 이와같이 얻은 방사능 물질을 정제, 확인하고 방사능 물질을 비교하여 본 결과 인삼사포닌은 뿌리에서, 당, ATP, NADPH 등을 잎으로부터 공급받아 합성되는 것으로 생각된다.

4. 잎에서는 주로 triol계 saponin과 당이 합성되고 줄기를 통해 뿌리로 공급하는 것으로 생각된다. 뿌리에서는 diol계 및 triol계 사포닌을 합성하나 diol계 사포닌이 많고 일부의 triol계 sapogenin 또는 saponin은 잎으로부터 줄기를 통해 공급받는 것으로 생각된다.

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