PLANT BIOCHEMISTRY OF GINSENG SAPONINS (III)

Radioactive Studies (2). Sodium Acetate-U-C^{14}, Feeding Experiment

Abstract

The radioactive compound sodium acetate-U-C^{14} (C-14 acetate) was administered to two- and four-year-old July and September American ginseng (*Panax quinquefolium* L.) plants and cuttings. The C-14 acetate uptake was approximately 99%. The autoradiochromatograms suggest that the saponins (panaquilins) isolated by preparative thin-layer chromatography contained impurities, especially those isolated from the leaf and stem extracts. The root and fruit methanol extracts yielded relatively pure saponins. The large amounts of panaquilin B and its proximity to panaquilin C on preparative thin-layer plates resulted in some admixing. The average concentration (% plant dry weight) of semi-purified saponins were high in the leaves (13.8%), compared to fruits (9.8%), stems (7.9%) and roots (6.3%). The average percentage of C-14 acetate incorporation into panaquilins was 4.8%. The average percentage of C-14 acetate incorporation into panaquilins B and C was higher (1.40% and 1.13%, respectively) than that into panaquilin C, (d), G-1 and G-2 (0.75%, 0.65%, 0.13% and 0.53%, respectively). Panaquilin synthesis may be depending upon the part collection period and age of the plant. The average percentage of C-14 acetate incorporation into panaquilin B is high in roots (0.58%) and stems (0.48%); that into panaquilins C and (d) high in leaves (0.40% and 0.45%, respectively); and that into panaquilin E high in roots and leaves (0.55% and 0.50%, respectively). Panaquilin G-2 was synthesized in all parts of plants. The panaquilins appear to be biosynthesized more actively in July than September (exception-panaquilin G-1). Panaquilins B, C and G-1 may be biosynthesized more actively in four-year-old plants and panaquilins (d) and E more actively in two-year-old plants. The results from expectance with cuttings suggest that the panaquilins are synthesized *de novo* in the above-ground parts of ginseng plants, and that panaquilin G-1 may be synthesized *de novo* in the leaf. It is known from the tissue culture studies that panaquilins are produced by leaf, stem and root callus tissues and callus-root cultures of American and Korean ginseng plants. Panaquilins may actively be synthesized *de novo* in most any cell or organ of the ginseng plants. It was verified that C-14 acetate was incorporated into the panaxadiol portions of the panaquilins of two-year-old plants (sp. act., 0.56 mμCi/mg) and four-year-old plants (sp. act., 0.54 mμCi/mg).

Introduction

The two previous reports considered the isolation, identification and distribution patterns of American ginseng saponins and sapogenins (1), and H^3-

squalene feeding experiments (2). In this study, the purpose of the radioisotope experiments, therefore, was to confirm and to quantitate the panaquilin tlc distribution patterns previously observed in plants not fed radioisotopes, and to obtain C^{14}-labeled panaquilins for future studies.

Results and Discussions

Radioactive sodium acetate-U-C^{14} (C-14 acetate) was fed by the wick method (3) to two-and four-year-old plants and cuttings at the beginning and the end of a growing season (July and September). After the feeding, the plants were allowed to grow in the field for 7 days, and the cuttings in a hydroponic solution for 5 days.

Aqueous C-14 acetate (400 mμCi/mg) solutions were fed to two-year-old plants (95 plants studied in July, 2.1 μCi/0.5ml/plant; 100 plants studied in September, 2 mμCi/0.5/plant), four-year-old plants (19 plants studied in July, 40 μCi/0.5ml/plant; 20 plants studied in September, 40 μCi/0.5ml/plant), two-year-old stem cuttings (40 cuttings studied in July 1μCi/0.5ml/cutting; 41 cuttings studied in September, 1 μCi/0.5ml/cutting) and four-year-old stem cuttings (20 cuttings studied in July, 10 μCi/0.5ml/cutting; 20 cuttings studied in September, 10 μCi/0.5ml/cutting; 20 cuttings studied in September 10 μCi/0.5 ml/cutting). The approximate fresh weights for two-year-old plants and cuttings were 3g and 1.5g, respectively; and for four-year-old plants and cuttings were 80g and 25g, respectively. The uptake of C-14 acetate solutions by two-and four-year-old plants was approximately 98.7%, and that for two-and four-old cuttings approximately 99.9%.

The amounts of extracts obtained from the plants and cuttings (Table I and II) are similar to those obtained from non-radioactive experiments (1). The thin-layer procedures used for isolating the saponins from radioactive extracts were the same as those used for non-radioactive extracts (1).

1. Extracts.

The percentages of ether extrcats were 1.5–8%; those of chloroform, 0.8–3.0%; and those of methanol-1, 20–40%. The total percentage of ether, chloro-

Table I. Extracts from Two-year-old Ginseng Plants and Cuttings: Sodium Acetate-U-C^{14} Experiments*.

a. Plants

	Leaf		Stem		Root		Average	
	Jl	Sp	Jl	Sp	Jl	Sp	Jl	Sp
Dry Wt. (g)	40.0	29.1	12.1	12.1	9.8	90.8	129.0	56.0
Extract(%)**								
Ether	13.0	5.5	1.1	4.6	1.4	1.3	5.2	3.8
Chloroform	1.8	2.7	0.2	0.7	0.4	1.7	0.8	1.7
Methanol-1	51.5	43.0	21.4	23.3	18.8	21.6	30.6	29.3
Residue	6.4	0.7	3.5	6.9	1.9	2.8	3.9	3.5
Methanol-2	40.9	42.3	17.8	16.4	16.9	18.8	25.2	35.8
Total	66.3	51.2	22.7	28.6	20.6	24.6	36.6	34.8
			b. Cuttings					
Dry Wt. (g)	16.5	20.6	4.5	7.8			10.5	14.2
Extract(%)**								
Ether	1.8	3.7	0.9	4.4			1.4	4.1
Chloroform	2.6	2.0	0.4	1.4			1.5	1.7
Methanol-1	39.3	54.5	13.8	25.4			28.1	40.0
Residue	5.2	NA	1.1	8.5			3.2	4.3
Methanol-2	25.8	48.3	12.7	16.8			19.3	32.3
Total	43.7	60.2	15.1	31.2			31.0	45.8

*Abbreviations: Jl-July Collection; Sp-September collection; NA-not available.

**Residue: Insoluble material of methanol-1 extracted with cold methanol (5°C); Methanol-2: Soluble extracts of methanol-1 extracted with cold methanol (5°C); Total = Ether (%) + chloroform (%) − methanol-1 (%).

Table II Extracts from Four-year-old Ginseng Plants and Cuttings: Sodium Acetate-U-C^{14} Experiments.*

a. Plants

	Leaf		Stem		Fruit		Root	
	Jl	Sp	Jl	Sp	Jl	Sp	Jl	Sp
Dry Wt. (g)	56.5	46.4	43.0	30.7	18.0	36.6	199.0	181.0
Extract (%)**								
Ether	8.9	2.9	2.9	0.8	5.2	15.8	1.5	0.5
Chloroform	2.0	1.7	0.3	0.8	0.7	9.5	0.9	0.4
Methanol-1	44.7	48.3	24.4	24.3	37.0	22.2	19.9	52.4
Residue	10.5	1.7	0.7	2.2	1.8	4.8	2.5	4.9
Methanol-2	27.4	28.3	23.7	22.1	35.3	17.3	17.3	47.5
Total	55.6	52.9	27.6	25.9	42.9	47.5	22.3	53.3
			b. Cuttings					
Dry Wt. (g)	45.0	49.4	32.2	32.6	9.4	39.1		
Extract (%)**								
Ether	3.3	5.0	1.2	1.3	3.7	17.2		
Chloroform	3.9	1.7	1.0	1.0	1.3	2.0		
Methanol-1	38.8	43.9	23.6	25.6	39.5	20.2		
Residue	4.6	0.1	0.4	1.9	2.3	5.1		
Methanol-2	30.4	38.8	23.2	23.7	37.2	15.1		
Total	46.0	50.6	25.8	27.9	44.5	39.4		

*Abbreviations: Jl-July collection; Sp-September collection.

**Residue: Insoluble material of methanol-1 extracted with cold methanol (5°C); Methanol-2: Soluble extracts of methanol-1 extracted with cold methanol (5°C); Total = Ether (%) + chloroform (%) + methanol-1 (%).

Table III. C^{14}-Activity (%) of Ether, Chloroform and Methanol Extracts*.

a. Plants

Plant	Two-year-old				Four-year-old			
Material**	Ether	Chloroform	Methanol	Total	Ether	Chloroform	Methanol	Total
JlL	0.1	0.3	15.4	15.8	0.02	0.3	9.9	10.2
JlS	2.8	0.4	8.2	11.4	0.9	0.3	5.2	6.4
JlF	NA	NA	NA	NA	0.3	0.1	2.0	2.4
JlR	0.6	0.2	3.6	4.4	0.4	0.1	3.0	3.5
Total	3.5	0.9	27.2	31.6	1.6	0.8	20.1	22.5
SpL	0.03	0.1	2.1	2.2	0.02	0.1	7.6	7.7
SpS	2.3	1.2	8.4	11.9	1.0	0.3	8.3	9.6
SpF	NA	NA	NA	NA	0.3	0.1	1.6	2.0
SpR	0.6	0.2	4.3	5.1	0.5	0.1	3.8	4.4
Total	2.7	1.5	14.8	19.2	1.8	0.6	21.3	23.4
			b. Cuttings					
JlL	0.7	0.8	23.8	25.3	NA	0.01	0.4	0.4
JlS	13.6	6.0	61.5	81.1	1.3	0.5	2.4	4.2
JlF	NA	NA	NA	NA	0.1	0.02	0.3	0.4
Total	14.3	6.8	85.3	106.4	1.4	0.5	3.1	5.0
SpL	0.1	0.3	14.8	15.2	0.01	0.1	3.6	3.7
SpS	3.5	1.2	16.6	21.3	2.1	0.5	16.6	19.2
SpF	NA	NA	NA	NA	0.3	0.1	1.6	2.0
Total	3.6	1.5	31.4	36.5	2.4	0.7	21.8	23.9

*C^{14}-Activity (%): (Total radioactivity in extract/Total radioactive uptake) × 100.

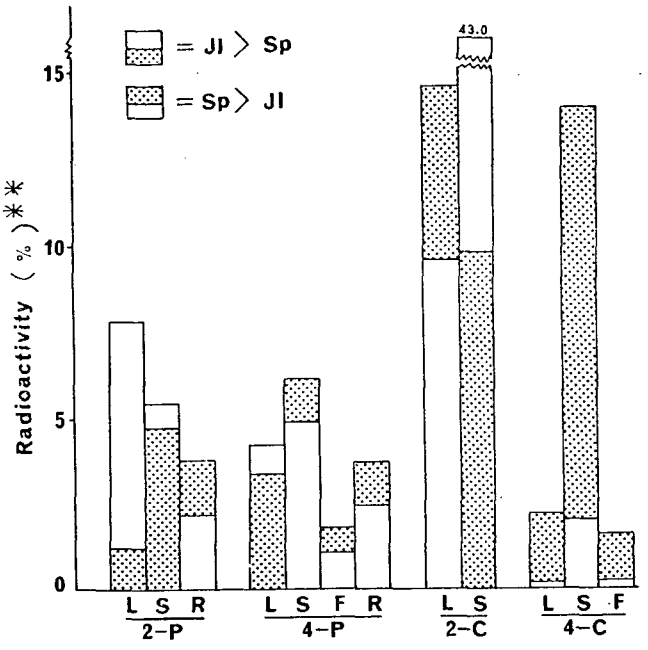
**Abbreviations: Jl-July collection; Sp-September collection; L-leaf; S-stem; F-fruit; R-root; NA-not available.

form and methanol-1 extracts were higher in the leaf than in the root (Table I and II). This result could be anticipitated because of the high amounts of pigments and lipids observed in the leaf extracts. The residue, data shown in Tables I and II, contains principally carbohydrates and is separated from methanol-1 by cold methanol (5°C).

The percentage of C-14 acetate incorporation into ether extracts was generally higher than that of chloroform extracts, but was lower than that of methanol extract-1 (Table III). The percentage of C-14 acetate incorporation into ether and chloroform extracts was consistantly higher in plants studied in July than those studied in September.

Methanol-2 saponin extracts were semi-purified in that considerable (2.4–4%) carbohydrate residue was removed (Tables I and II). The percentage of methanol-2 C-14 acetate incorporation is less (average 26.1%) than that in methanol-1 (average 33.1%), and is probably due to its incorporation into carbohydrates (Table III and Fig. 1). The percentage of

Figure 1. Radioactivity of Methanol-2 Extracts: C^{14}-Experiments*.

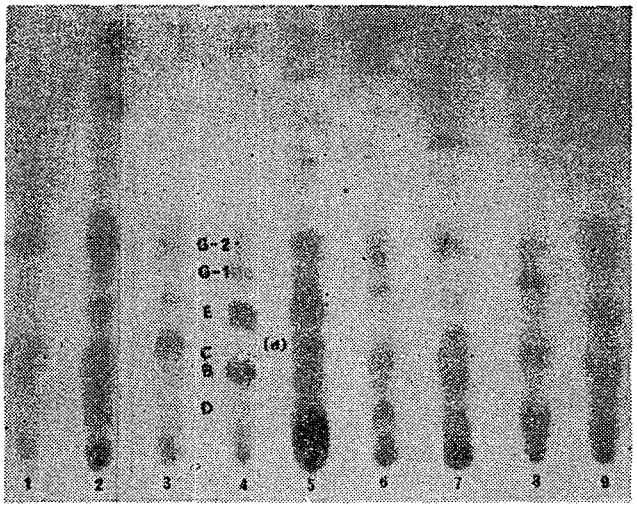


* Abbreviations: L-leaf; S-stem; F-fruit; R-root; 2-P: Two-year-old plants; 4-P: Four-year-old plants; C: Cuttings; Jl-July collection; Sp-September collection.

**Radioactivity (%): (Total radioactivity in extract/Total radioactive uptake)X100.

methanol-2 C-14 acetate incorporation is high (15.4%) in two-year-old plants collected in July (Fig. 1). The average percentage of C-14 acetate incorporation is low in fruits (1.5%) as compared to roots (3.1%), leaves (4.2%) and stems (6.2%). The average percentage of C-14 acetate incorporation was higher in roots studied in September (3.8%) than those studied in July (2.4%). The leaves studied in July had a higher average C-14 acetate incorporation than the roots (6.1% and 2.3%, respectively), but the leaves

Plate 1. One-dimensional Autoradiochromatography of American Ginseng Methanol-2 Extracts*.



D-panaquilin D; B-panaquilin B; C-panaquilin C; (d)-panaquilin (d); E-panaquilin E; G-1-panaquilin G-1; G-2-panaquilin G-2.

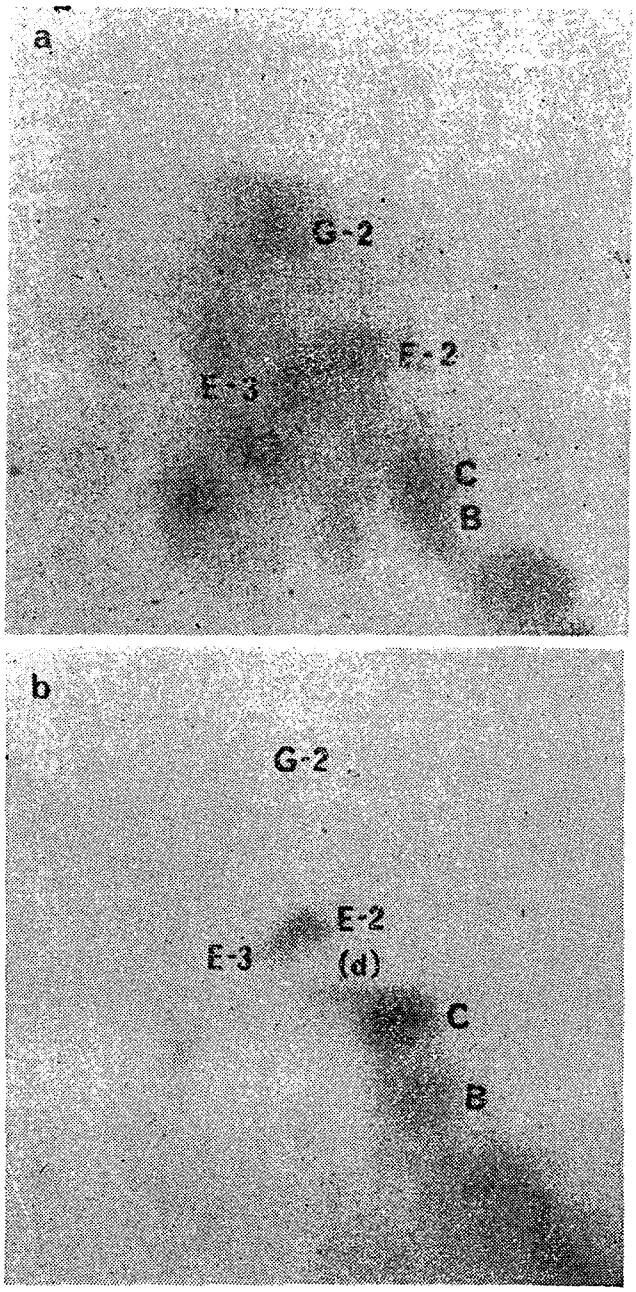
Film: Kodak RP Royal X-Omat (Eastman Kodak Co., Rochester, N. Y.); *Development*: Kodak X-ray developer; *Exposure time*: 42 days.

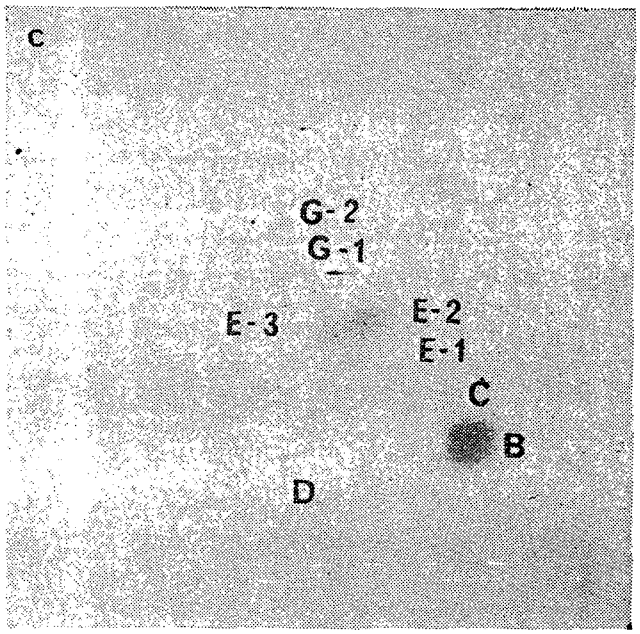
Samples: 1. Four-year-old leaves collected in July (cutting, 20 lambda, 70 cpm); 2. Four-year-old stems collected in July (cutting, 20 lambda, 1,540 cpm); 3. Four-year-old fruits collected in July (cutting, 40 lambda, 520 cpm); 4. Two-year-old roots collected in July (30 lambda, 1,340 cpm); 5. Two-year-old leaves collected in July (cutting, 40 lambda, 2,980 cpm); 6. Four-year-old leaves collected in September (cutting, 20 lambda, 800 cpm); 7. Four-year-old fruits collected in September (cutting, 40 lambda, 1,340 cpm); 8. Two-year-old leaves collected in September (20 lambda, 760 cpm); 9. Two-year-old roots collected in September (40 lambda, 1,980 cpm).

Silica gel plate: 20 × 20 cm; thickness: 0.5 mm; solvent system; chloroform: methanol: distilled water = 65:35:10.

*The background of original autoradiochromatograms negative was darker than that of this Plate, and panaquilins (d), G-1 and G-2 were visible in the original, but are not visible in the Plate.

Plate 2. Two-dimensional Autoradiochromatograms of American Ginseng Methanol-2 Extracts from Four-year-old Plants Collected in July*.





a. Leaf extracts; 20 lambda (6,120 cpm), 14 days exposure. Panaquilins B, C, E-2, E-3 and G-2.
b. Fruit extracts; 20 lambda (5,300 cpm), 14 days exposure. Panaquilins B, C, (d), E-2, E-3 and G-2.
c. Root extracts; 20 lambda (1,870 cpm), 28 days exposure. Panaquilins B, C, D, E-1, E-2, E-3, G-1 and G-2.
*The original background of autoradiochromatograms negative was darker than that of this Plate, and panaquilins (d), G-1 and G-2 were visible, but are not visible in the Plate.

studied in September were lower than the roots (2.4% and 3.8%, respectively).

When extract activity was expressed as μCi per mg dry weight it was consistantly higher in stems (7.47 mμCi/mg) and fruits (1.03 mμCi/mg) than in leaves (0.83 mμCi/mg) and roots (0. .38 Ci/mg). This is probably related to the fact that the stem wick method was used to administer the radioactive compound.

In cuttings, the percentage of C-14 acetate incorporation into the methanol-2 leaf extracts of two-year-old cuttings was higher in September (14.6%) than July (9.5%). There was significant amounts of methanol-2 extract in two- and four-year-old July green immature fruits (average 37.8%) as compared to the September mature fruits (average 16.2%) (Table II).

2. Autoradiochromatography of Methanol-2.

The radioactive methanol-2 solutions (approximately 10% in methanol, 20–60 lambda) were applied to silica gel plate (0.5 mm thick). The plates were exposed to X-ray films (Kodak RP Royal X-Omat) for 1–6 weeks depending on the activity (100–37,000 cpm) of the aliquot applied. Radioactive saponin patterns (Plates 1 and 2) were similar to non-radioactive saponin patterns verifying C-14 acetate incorporation into saponins.

3. Total Radioactivity of Semi-purified Saponins.

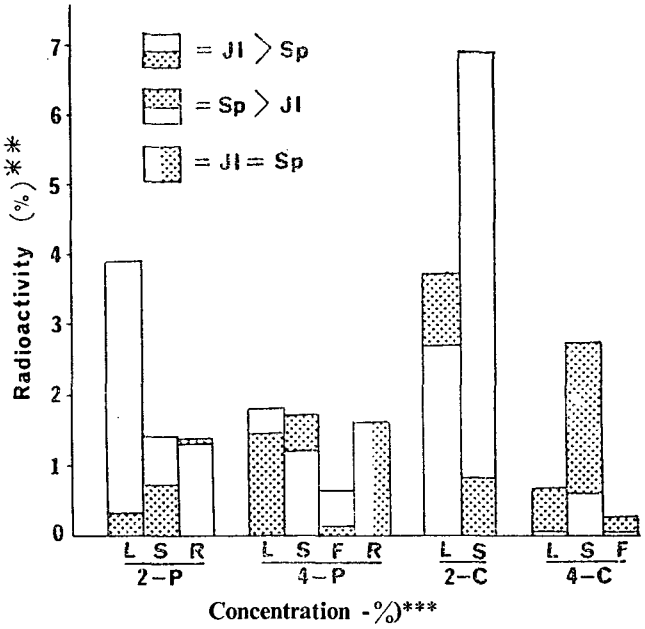
Semi-purified saponins were obtained from silica gel plates by eluting their zone with methanol. The semi-purified saponin concentration show in Fig. 2 is based on the dry weight total of all the saponin band elutes. The semi-purified saponins did not contain visible silica gel residues. The five bands eluted and studied were panaquilin B (rf-value, 0.23), panaquilin C (rf-value, 30), panaquilin E (rf-value, 0.41), panaquilin G-1 (rf-value, 0.55) and panaquilin G-2 (rf-value, 0.63) (1). The large amount of panaquilin B and its rf proximity to panaquilin C resulted in some admixing. The autoradiochromatography also suggests that saponin bands may have contained impurities, especially those isolated from the leaf and stem extracts (Plate 2). The root and fruit methanol extracts contained relatively pure saponins.

a. Plants

The total percentage of C-14 acetate incorporation into the semi-purified saponins (Fig. 2) of two-year-old plants collected in July (6.6%) was higher than that incorporated into September collected plants (2.4%), and the percentage incorporated into the semi-purified saponins of four-year-old plants collected in July (5.4%) was slightly higher than that in September collected plants (4.8%). The average percentage of C-14 acetate incorporated into root saponins of July collected plants (1.45%) was approximately the same as September collected roots (1.50%). The radioactivity (%) data suggests that the physiological activity of two-year-old leaves with regard to C-14 acetate incorporation into saponins is significantly higher in July than in September, and that the roots maintain a fairly constant physiological activity (Fig. 2). The percentage of C-14 acetate incorporation in two-year-old plants collected in September (2.4%) was lower than that at any other time studied.

The concentration (%) of semi-purified saponins were similarly high (average 1.38%) in both July and September leaves (Fig. 2). This observation is significant, as it is generally believed that the saponins existed principally or only in the root. There is but one related publication (4) which states the presence of ginseng sapogenins panaxadiol, panaxatriol

Figure 2. Semi-purified Saponin C-14 Radioactivity Distribution and Concentration (%) in American Ginseng Plants and Cuttings*.



	2-P			4-P				2-C		4-C		
	L	S	R	L	S	F	R	L	S	L	S	F
Jl	15.1	6.7	10.1	9.8	4.5	13.1	5.4	11.8	3.4	21.4	9.2	19.4
Sp	16.0	5.2	4.2	14.3	15.2	6.5	5.5	15.7	4.8	16.8	5.8	6.4

* Abbreviations: L-leaf; S-stem; F-fruit; R-root; Jl-July collection; Sp-September collection; 2-P: Two-year-old plants; 4-P: Four-year-old plants; C-cuttings.

** Radioactivity (%): (Radioactivity) saponins (μCi)/Radioactivity uptake (μCi)) × 100.

***Concentration (%): (Weight of total saponins (mg)/ Weight of plants (mg dry weight)) × 100.

and oleanolic acid in the above-ground parts of *Panax ginseng*. The semi-purified saponin content of roots collected in July was at least twice as high (10.1%) as that collected in September (4.2%) for two-year-old roots. The content of semi-purified saponins from the matured red-ripened September fruits was approximately twice lower (6.5%) than that from the immature green July fruits (13.1%).

b. Cuttings.

The radioactivity (%) of semi-purified saponins was high and localized in the stems. The unusually high localization of radioactivity in July collected stems may be related to their fragile and young structure and the fact that the stem wick method of radioisotope administration was employed. The average size of the two-year-old stems was 1–2 mm in diameter and 5–7 cm in stem height. Although the radio-

activity (%) was generally lower than that in stem, it was present in both leaf and fruit crude saponin fractions.

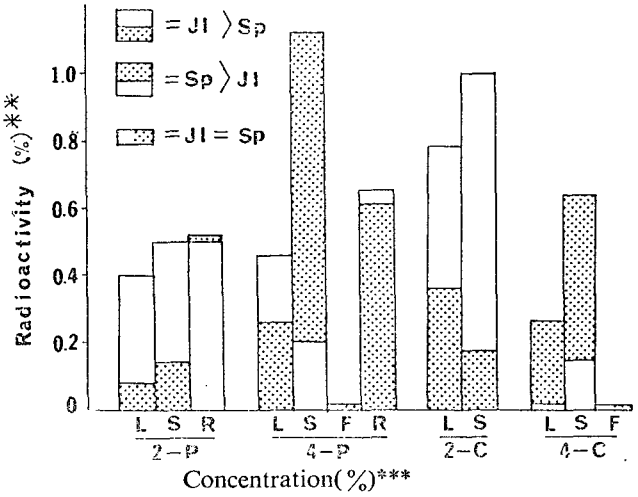
4. Radioactivity of Semi-purified Saponins.

a. Panaquilin B (Panaxadiol Genin).

(1) Plants.

The percentage of C-14 acetate incorporated into panaquilin B by the plant was higher in July than in September, with the exception of two-year-old roots and four-year-old stems (Fig. 3). Four-year-old plants contained more panaquilin B activity (1.8 %) than two-year-old plants (1.1 %). Panaquilin B activity was low in four-year-old July and Setpember fruits (0.05%). The average panaquilin B activity was consistantly higher in two- and four-year-old roots (0.6%) than that in leaves (0.3%).

Figure 3. Panaquilin B C-14 Radioactivity Distribution and Concentration (%) in American Ginseng Plants and Cuttings*



	2-P			4-P				2-C		4-C		
	L	S	R	L	S	F	R	L	S	L	S	F
Jl	2.0	2.6	2.6	2.6	2.2	4.2	2.6	4.1	1.8	4.5	2.2	6.1
Sp	3.5	2.2	1.2	4.4	6.2	1.5	2.5	5.3	1.7	5.7	2.1	1.5

* Abbreviations: L-leaf; S-stem; F-furit; R-root; Jl-July collection; Sp-September collection; 2-P: Two-year-old plants; 4-P: Four-year-old plants; C-cuttings.

** Radioactivity (%): (Radioactivity in panaquilin B (μCi)/Radioactivity uptake (μCi) × 100.

***Concentration (%): (Weight of panaquilin B (mg)/ Weight of plants (mg dry weight)) × 100.

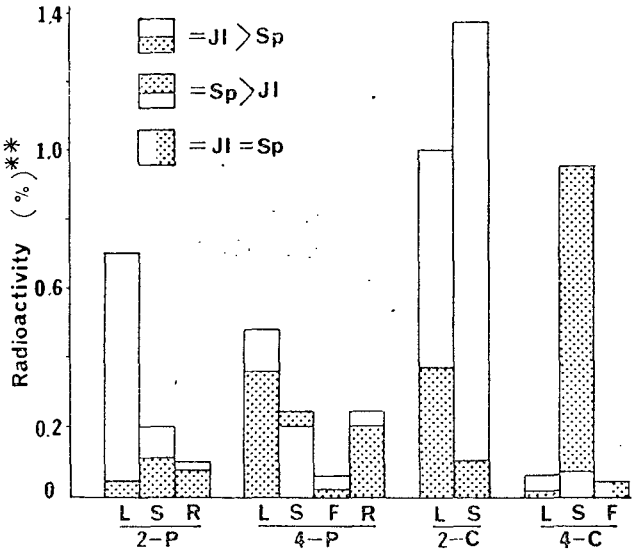
The average concentration of panaquilin B in July collected roots was higher (2.6%) than that in September collected roots (1.7%). The above-ground parts (leaves and stems) contained significant amounts of panaquilin B in two-year-old (average 5.2%) and four-year-old plants (average 7.7%). Four-year-old stems collected in September contained the highest percentage of panaquilin B (6.2%), and perhaps this observation relates to its lower concentration in mature fruits (1.5%) and immature fruits (4.2%).

(2) Cuttings.

The total percentage of C-14 acetate incorporation was high in July in two-year-old cuttings (1.8%), and high in September in four-year-old cuttings (1.2 %). The higher percentage of panaquilin B activity in four-year-old September stems (0.8%) than that in four-year-old July stems (0.1%) may be related to fruit maturation.

b. Panaquilin C (Panaxadiol Genin).

Figure 4. Panaquilin C C-14 Radioactivity Distribution and Concentration (%) in American Ginseng Plants and Cuttings*



	2-P			4-P				2-C		4-C		
	L	S	R	L	S	F	R	L	S	L	S	F
Jl	4.0	1.3	0.8	3.0	0.9	5.2	0.6	2.9	0.5	6.2	1.9	7.7
Sp	5.6	1.3	0.4	3.1	3.8	2.5	1.0	3.7	1.3	4.1	1.4	2.4

* Abbreviations: L-leaf; S-stem; F-fruit; R-root; Jl-July collection; Sp-September collection; 2-P: two-year-old plants; 4-P: four-year-old plants; C:cuttings.

** Radioactivity (%): (Radioactivity in panaquilin C (μCi)/Radioactivity uptake (μCi) × 100.

***Concentration (%): (Weight of panaquilin C (mg)/ weight of plants (mg dry weight)) × 100.

(1) Plants.

The percentage of C-14 acetate incorporation into panaquilin C was with one exception (four-year-old stems) higher in July than in September (Fig. 4). The average panaquilin C activity in the roots (0.15%) was generally lower than that of leaves (0.4%) or stems (0.18%). Four-year-old roots contained more panaquilin C activity (0.2%) than two-year old roots (0.1%). With the exception of two-year-old September leaves (0.03%), the leaves contained the highest percentage of panaquilin C activity (average 0.5%). As with panaquilin B, higher panaquilin C activity in September stems may be related to fruit maturation.

Panaquilin C concentration (%) was highest in two-year-old September leaves (5.6%), and four-year-old July fruits (5.2%). The high concentration of panaquilin C present in immature fruits (5.2%) may be related to its low concentration in the stems (0.9%).

(2) Cuttings.

The percentage of C-14 acetate incorporation into panaquilin C in two-year-old cuttings collected in July was higher (2.4%) than in September (0.5%). Four-year-old cuttings collected in September contained more panaquilin C activity (1.0%) than those collected in July (0.1%). The unusually high four-year-old stem panaquilin C activity may be related to fruit maturation.

c. Panaquilin (d) (Unknown Genin).

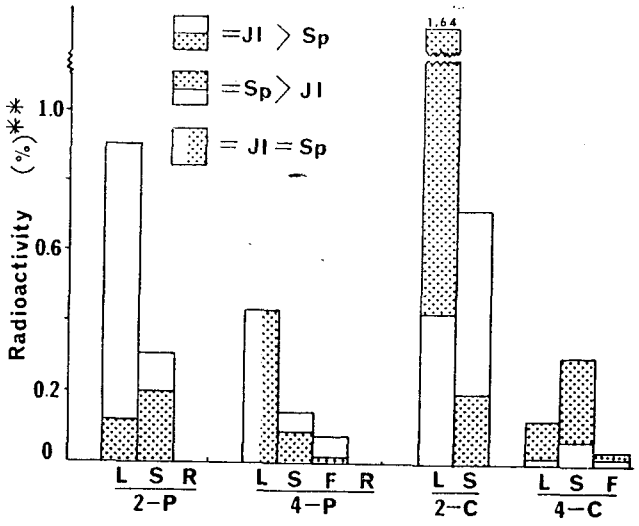
(1) Plants.

Panaquilin (d) was not present in American ginseng root. This confirms the two-dimensional thin-layer chromatography observation made with non-radioactive materials. Also, panaquilin (d) was absent from autoradiochromatograms (Plates 1 and 2).

The average percentage of C-14 acetate incorporation into panaquilin (d) was highest in two-year-old leaves collected in July (0.9%), and was in general higher in the leaves (0.5%) than in the stems (0.2%) and fruits (0.05%) (Fig. 5). The average panaquilin (d) activity was also higher in July collected two- and four-year-old plants (0.6% and 0.2%, respectively) than in September collected plants (0.15% and 0.17%, respectively).

Panaquilin (d) concentration (%) was higher in

Figure 5. Panaquilin (d) C-14 Radioactivity Distribution and Concentration (%) in American Ginseng Plants and Cuttings*.



Concentration (%)***

	2-P			4-P				2-C		4-C		
	L	A	R	L	S	F	R	L	S	L	S	F
Jl	2.9	0.5	–	1.8	0.3	1.2	–	2.7	0.3	4.3	1.2	1.9
Sp	2.3	0.4	–	2.4	1.8	1.2	–	1.5	0.7	1.4	0.8	1.2

* Abbreviations: L-leaf; S-stem; F-fruit; R-roots; 2-P: two-year-old plants; 4-P: four-year-old plants: C-cuttings; Jl-July collection; Sp-September collection.

** Radioactivity (%): (Radioactivity in panaquilin (d) (μCi)/Radioactivity uptake (μCi)) × 100.

***Concentration (%): (Weight of panaquilin (d) (mg)/ Weight of plants (mg dry weight)) × 100.

the leaves of the plants (2.4%) as compared to its stems (0.8%) or fruits (1.2%). The concentration of panaquilin (d) in July immature fruits is the same (1.2%) as that in September mature fruits.

(2) Cuttings.

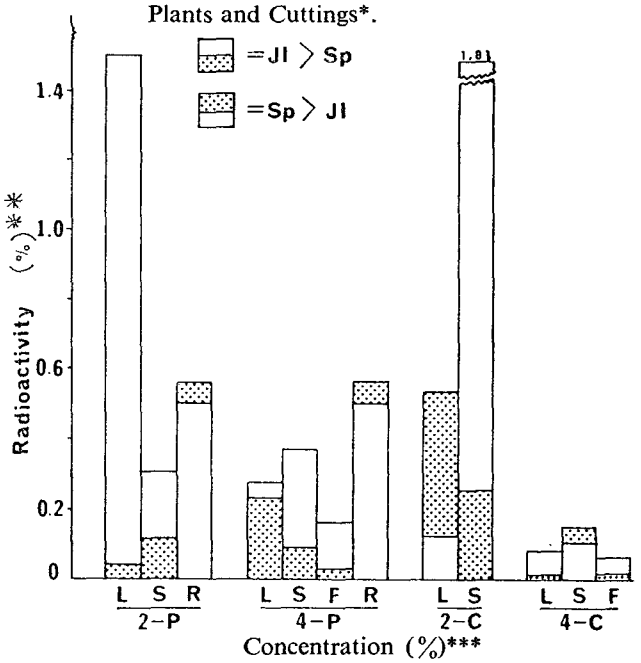
The average percentage of C-14 acetate incorporation into panaquilin (d) was higher in September (0.44%) than in July (0.24%), and in two-year-old cuttings (0.73%) than four-year-old cuttings (0.09%) The average concentration of panaquilin (d) was consistantly higher in the leaves (2.5%) than in the stems (0.75%) or fruits (1.6%). The average concentration of panaquilin (d) in immature fruits (1.9%) is slightly higher than that in September fruits (1.2%)

d. Panaquilin E (Panaxadiol and Panaxatriol Genins).

(1) Plants.

The average percentage of C-14 acetate incor-

Figure 6. Panaquilin E C-14 Radioactivity Distribution and Concentration (%) in Ammerican Ginseng Plants and Cuttings*.



	2-P			4-P				2-C		4-C		
	L	S	R	L	S	F	R	L	S	L	S	F
Jl	3.3	0.6	2.1	2.1	0.6	1.6	1.7	1.3	0.2	4.6	2.8	2.2
Sp	3.8	0.5	1.9	3.4	1.9	0.8	1.5	3.0	0.4	3.6	0.6	0.8

* Abbreviations: L-leaf; S-stem; F-fruit; R-root; 2-P: two-year-old plants; 4-P: four-year-old plants; C-cuttings; Jl-July collection; Sp-September collection.

** Radioactivity (%): (Radioactivity in panaquilin E (μCi)/Radioactivity uptake (μCi)) × 100.

***Concentration (%): (Weight of panaquilin E (mg)/ Weight of plants (mg dry weight)) × 100.

poration into panaquilin E in plants collected in July was higher (1.9%) than those collected in September (0.8%) (Fig. 6). With the exception of two-year-old July collected leaves (1.5%), the panaquilin E activity in the above-ground parts was consistantly lower than that in the roots (0.55%). The panaquilin E activity in July collected roots was slightly lower (0.5%) than that in September collected roots (0.6%).

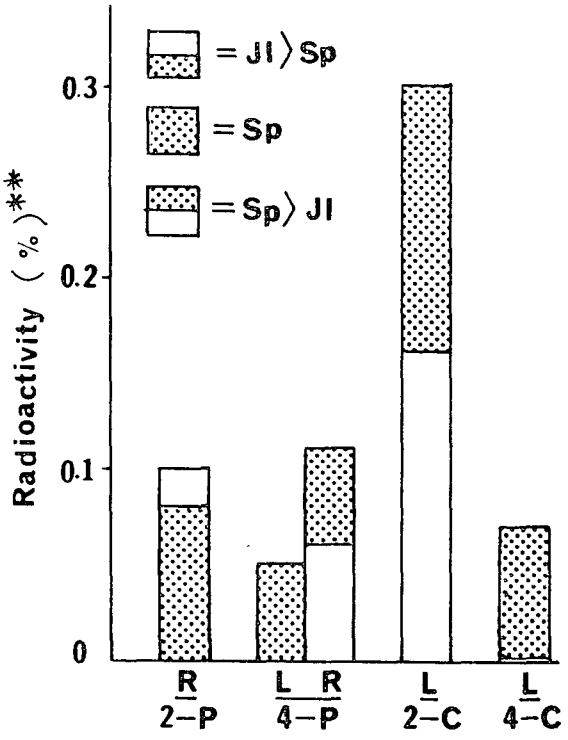
The average concentration of panaquilin E in the roots collected in July was higher (1.9%) than that in September (1.7%), and four-year-old roots (2.0%). The leaf (average 3.2%) consistantly contained more panaquilin E than the root (average 1.8%). In four-year-old plants, July collected stems (0.6%) and September mature fruits (0.8%) contained less panaquilin E than the green immature fruits (1.6%).

(2) Cuttings.

The average percentage of C-14 acetate incorporation into panaquilin E in two-year-old cuttings collected in July was higher (1.0%) than that in September cuttings (0.4%). The average concentration (%) of panaquilin E were high in four-year-old cuttings (2.4%) as compared to that in two-year-old cuttings (1.2%). The concentration of panaquilin E in July collected green immature furits was higher (2.2%) than that in September collected mature fruits (0.8%).

e. Panaquilin G-1 (Panaxatriol Genin).

Figure 7. Panaquilin G-1 C-14 Radioactivity Distribution and Concentration (%) in American Ginseng Plants and Cuttings*.



	2-P	4-P		2-C	4-C
	R	L	R	L	L
Jl	2.4	-	0.3	0.4	0.2
Sp	0.3	0.1	0.1	1.1	0.8

* Abbreviations: L-leaf; S-stem; F-fruit; R-root; Jl-July collection; Sp-September collection; 2-P: two-year-old plants; 4-P: four-year-old plants; C-cuttings.

** Radioactivity (%): (Radioactivity in panaquilin G-1 (μCi)/Radioactivity uptake (μCi)) × 100.

***Concentration (%): (Weight of panaquilin G-1 (mg)/ Weight of plants (mg dry weight)) × 100.

(1) Plants.

The average percentage of C-14 acetate incorporation into panaquilin G-1 was similar (0.09%) in both two- and four-year-old roots (Fig. 7). Panaquilin G-1, in both the labeled and non-labeled form, was absent from the stems and leaves (exception-leaves from four-year-old plants). The panaquilin G-1 concentration was approximately 8 times more (2.4%) in two-year-old roots collected in July than September (0.3%).

The data suggests that panaquilin G-1 may be synthesized *de novo* in either the leaf and root, or may be present and translocated in some other chemical forms through the stems.

(2) Cuttings.

The two-year-old leaves collected from July and September cuttings contained radioactive panaquilin G-1. In four-year-old cuttings, the July collected leaves contained only trace amounts of panaquilin G-1 (0.002%). Panaquilin G-1 was not present in the stems of the cuttings.

f. Panaquilin G-2 (Unknown Genin).

(1) Plants.

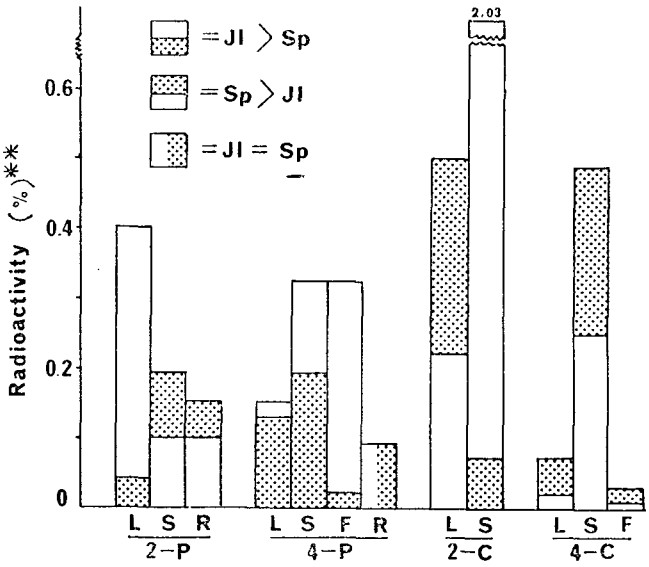
The average percentage of C-14 acetate incorporation into panaquilin G-2 was high (0.3%) in two- and four-year-old leaves collected in July as compared to two- and four-year-old leaves collected in September (0.07%) (Fig. 8). Panaquilin G-2 activity was particularly high in two-year-old leaves collected in July (0.4%) and in four-year-old July stems (0.3%) and fruits (0.3%). The average panaquilin G-2 activity in two-year-old roots (0.15%) was higher than that in four-year-old roots (0.10%).

The concentration of panaquilin G-2 was highest in July collected plants (2.3%). The immature (0.9%) and mature (0.5%) fruits also contained panaquilin G-2. The leaves contained more panaquilin G-2 than the roots (average 1.2% and 0.8%, respectively).

(2) Cuttings.

The average percentage of C-14 acetate in panaquilin G-2 in two-year-old cuttings (1.4%) was higher than that of four-year-old cuttings (0.5%). The stems collected in July contained a very high percentage of panaquilin G-2 activity (2.0%). Except for these stems, the percentage into panaquilin G-2

Figure 8. Panaquilin G-2 C-14 Radioactivity Distribution and Concentration (%) in American Ginseng Plants and Cuttings*.



	2-P			4-P				2-C		4-C		
	L	S	R	L	S	F	R	L	S	L	S	F
Jl	2.9	1.7	2.2	0.2	0.5	0.9	0.2	0.4	0.6	1.8	1.1	1.5
Sp	0.8	0.8	0.4	0.9	1.5	0.5	0.4	1.1	0.7	1.2	0.9	0.5

* Abbreviations: L-leaf; S-stem; F-fruit; R-root; Jl-July collection; Sp-September collection; 2-P: two-year-old plants; 4-P: four-year-old plants; C-cuttings.

** Radioactivity (%): (Radioactivity in panaquilin G-2 (μCi)/Radioactivity uptake (μCi)) × 100.

***Concentration (%): (Weight of panaquilin (mg)/Weight of plants (mg dry weight)) × 100.

Table IV. Panaquilin Radioactivity Distribution in American Ginseng Plants*.

Plant	Panaquilin						
	B	C	(d)	E	G-1	G-2	Total
Part							
Leaf	0.33	0.40	0.45	0.50	0.10	0.18	4.50
Stem	0.48	0.18	0.18	0.23	—	0.20	1.28
Fruit	0.05	0.05	0.10	—	—	0.15	0.40
Root	0.58	0.15	—	0.55	0.10	0.13	1.48
Collection							
July	1.45	1.00	0.90	1.85	0.10	0.70	6.00
September	1.35	0.50	0.40	0.80	0.15	0.35	3.60
Age							
Two-year-old	1.05	0.60	0.75	1.50	0.10	0.50	4.50
Four-year-old	1.75	0.90	0.55	1.15	0.15	0.55	5.10

*Radioactivity (%): (Radioactivity in panaquilins (μCi)/radioactivity C-14 acetate uptake by plant (μCi) × 100. Average of four studies (July and September collections from two-and four-year-old plants).

Table V. Hydrolysates of Methanol-2: C^{14}-Activity.

a. Two-year-old

Plant Material*	Methanol-2**		Hydrolysates***		Ratio****
	Aliquot (g)	Activity (mμCi/mg)	Amount (g)	Activity (mμCi/mg)	
JlA	1.16	1.58	0.21	4.53	2.9
JlR	1.03	0.33	0.20	1.14	3.5
Average	1.10	0.96	0.21	2.84	3.2
SpA	1.10	0.69	0.33	1.01	1.5
SpR	0.96	0.41	0.23	0.76	1.9
Average	1.03	0.55	0.28	0.89	1.7
SpA (C)	1.06	1.04	0.30	1.54	1.5
		b. Four-year-old			
JlA	1.11	2.52	0.22	6.04	2.4
JlR	1.14	0.57	0.27	1.72	3.0
Average	1.13	1.55	0.25	3.88	2.7
SpA	1.09	2.95	0.23	6.13	2.1
SpR	1.14	0.37	0.09	2.10	5.7
Average	1.12	1.66	0.16	4.12	3.8
Sp (C)	1.11	1.21	0.26	1.70	1.4

*Abbreviations: Jl-July collection; Sp-September collection; A-above-ground parts; R-root; C-cutting.
**Methanol-2: Soluble extracts of methanol-1- with cold methanol (5°C).
***Hydrolysates: Ether extracts of hydrolysates of methanol-2 with 30% hydrochloric acid and methanol (1:4).
****Ratio: Hydrolysate activity/Methanol-2 activity.

were higher in September cuttings (0.6%) than July cuttings (0.3%).

The concentration (%) of panaquilin G-2 was low in two-year-old cuttings collected in July (1.0%) as compared to two-year-old cuttings collected in September (1.8%) or four-year-old cuttings (3.5%). A relatively high concentration of panaquilin G-2 was present in the immature fruits (1.5%).

g. Summary.

The results of C-14 acetate incorporation into each panaquilin are summarized in Table IV.

5. Hydrolysis of Radioactive Methanol-2

a. Radioactivity of Hydrolysates.

Aliquots of radioactive methanol-2 extracts (0.96–1.16 g, activity 0.37–2.52 mμCi/mg) (Table V) were hydrolyzed with a mixture of methanol and 30 % hydrochloric acid (4:1) by refluxing on a steam bath for 5 hrs. The hydrolyzed solution was evaporated and the residue was extracted with ether. The ether extracts weighed 0.09–0.30 g, were radioactive 0.76–6.13 mμCi/mg, and contained ginseng genins and probably impurities such as fatty acids and flavonoids.

The average activity (mμCi/mg) of hydrolysates in the above-ground parts of plants (4.43 mμCi/mg) was higher than that in the roots (1.43 mμCi/mg). In cuttings, the hydrolysates from the above-ground parts contained approximately 1.60 mμCi/mg. The average activity of hydrolysates was lower in two-year-old plants (1.87 mμCi/mg) than that in four-year-old plants (4.00 mμCi/mg). The average activity of hydrolysates in four-year-old plants (4.00 mμCi/mg) was higher than either two-year-old plants collected in July (2.84 mμCimg) or September (0.89 mμ Ci/mg).

The ratio of hydrolysate actvity to methanol-2 activity was 1.4–5.7 (Table V). The ratio was lower in the above-ground parts (average 2.2) than in the root (average 3.5). This may indicate that the root contained more ether-soluble hydrolysates or less impurities, such as pigments and carbohydrates than the above-ground parts. The ratio (average) is higher in two-year-old plants collected in July (3.2) than in September (1.7), whereas the ratio is lower in four-year-old plants collected in July (2.7) than in September (3.9).

b. Radioactivity of Panaxadiol.

Table VI. Dilution and Recrystallization of C-14 Panaxadiol.

a. Two-year-old

Plant Material	Panaxadiol** Amount (mg)	Panaxadiol** Activity (mμCi/mg)	Panaxadiol Added*** (mg)	Purified (1x)**** Amount (mg)	Purified (1x)**** Activity (mμCi/mg)
JlA	23	2.77	15	18	0.91
JlR	11	1.29	10	15	0.54
SpA	67	0.42	10	6	0.10
SpR	48	0.67	11	24	0.49
		b. Four-year-old			
JlA	14	2.96	12	13	0.91
JlR	9	2.23	17	19	0.65
SpA	42	1.11	15	17	0.23
SpR	15	1.93	11	15	0.98

*Abbreviations: Jl-July collection; Sp-September collection; A-Above-ground; R-root.
**Panaxadiol: Radioactive panaxadiol fractions obtained from preparative tlc bands.
***Panaxadiol Added: Non-radioactive panaxadiol addition to radioactive panaxadiol fraction (mg).
****Purified (1x): First recrystallization radioactive panaxadiol.

Radioactive panaxadiol (0.42–2.96 mμCi/mg, 9–67 mg) was isolated from the ether-soluble extracts of hydrolysates by methanol elution of its silica gel tlc band (Table VI). This radioactivity was lower than that of he original ether extracts of the hydrolysate (0.67–6.13 mμCi/mg). The radioactive panaxadiol obtained from tlc could not be perfectly recrystallized, probably due to the presence of impurities. Non-radioactive panaxadiol (10–17 mg) was added to the panaxadiol (9–67 mg), and crystallized (Purified (IX): 6–24 mg, Table VI).

The average radioactivity of the combined, purified (IX) panaxadiol was 0.51 mμCi/mg in two-year-old plants and 0.69 mμCi/mg in four-year-old plants (Table VI). After three additional recrystallizations, the specific activity of the panaxadiol from two-year-old plants was 0.56 mμCi/mg and 0.54 mμCi/mg from four-year-old plants.

Experimental

The analytical procedures for the radioactive panaquilin and panaxadiol were essentially identical to that have been described (1,2) except for the following:

1. Isotope Materials.

Sodium acetate-U-C^{14} (1 mCi/1 ml, 102 mCi/mM, Lot No. C 32711) was purchased from Dhom Products, Ltd., North Hollywood, California. Toluene-C^{14} ($5.67 \times 10^5 \pm 3\%$ dpm/g) for isotope standard was purchased from the Packard Instrument Co., Inc., Downers Grove, Illinois.

2. Feeding of Sodium Acetate-U-C^{14} to Intact Plants.

An aqueous labeled acetate stock solution (10 ml) was prepared (100 μCi/mM) by adding non-radioactive sodium acetate (200 mg) to sodium acetate-U-C^{14} (1.0 ml, 1 μCi). Aliquots of the stock solution were then used to prepare feeding solutions (1 μCi/0.5 ml; 10 μCi/0.5 ml; 40 μCi/0.5 ml).

Tracer solutions (10 μCi/10–20 fresh plant weight) were then fed to 100 two- (1 μCi/0.5 ml) and 20 four-year-old (40 μCi/0.5 ml) plants by the wick method (3). The plants took up the radioactive material in approximately 2 hrs. The tracer solution vials were washed with distilled water (0.2 ml) and the resulting solution was also taken up by the plant. The washing procedure was again repeated. The following day the thread and vials were removed from the plants and bamboo supports provided for plant. After one week, the plants were collected Approximately 0.1–0.15% of the radioactivity remained in the thread and vials.

3. Feeding of Sodium Acetate-U-C^{14} to Stem Cuttings.

Labeled acetate solutions were fed by the wick method to 40 two-year-old stem cuttings (1 μCi/0.5 ml) and to 20 four-year-old stem cuttings (10 μCi/0.5 ml). The stem cuttings were maintained in Hyponex solution (7–6–19, Hydroponic Chemical Co., Inc., Copley, Ohio). Approximately 0.004–0.06% of the radioactivity remained in the thread and vials. The plants were collected for analysis after 5 days.

4. Autoradiochromatography (5).

Kodak RP Royal X-Omat and Liquid X-ray develop were used (Eastman Kodak Co., Rochester, N. Y.). The radioactivity of samples and standards was determined prior to their application to tlc plates to more properly estimate the X-ray film exposure time. The thin layer chromatogram was placed directly on an unexposed X-ray film and placed in a cardboard X-ray cassette. The cassettes were covered with a heavy steel plate (approximately 1.5 kg) to maintain uniform contact between the film and plate, and the film exposed for the required time. Developed X-ray films were examined against a white background. The thin-layer chromatograms used to prepare the X-ray films were then sprayed with a ceric sulfate solution, and the results compared.

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