

The allergens of *Dendropanax trifidus* Makino and *Fatsia japonica* Decne. et Planch. and evaluation of cross-reactions with other plants of the Araliaceae family

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cis-9,17-Octadecadiene-12,14-diyne-1,16-diol (**I**), an analog of falcarinol has been identified in our previous report as an active component of *Dendropanax trifidus* and a strong sensitizer. In this report, 16-hydroxy-cis-9,17-octadecadiene-12,14-diyne-1,16-diol (**II**) and cis-9,trans-16-octadecadiene-12,14-diyne-1,16-diol (**III**) were elucidated as 2 other active components of the plant. Compound **I**, however, presented with the highest concentration and showed a stronger reaction on patch testing. The leaves of *Fatsia japonica* Decne. et Planch. were also found to contain compound **I**, but the amount was found to be 7× more in *Dendropanax trifidus* than in *Fatsia japonica*. 5 subjects with hypersensitivity to *Dendropanax trifidus* and compound **I** showed positive reactions when patch tested with the leaves of *Hedera helix* L. and *Schefflera arboricola*. 1 of these also showed a positive reaction to the extract of *Panax ginseng* root powder diluted 1% in ethanol. There was cross-reaction among these plants, which all belong to the Araliaceae family.

Key words: phytocontact allergy; cross-reaction; *Dendropanax trifidus*; *Fatsia japonica*; *Hedera helix*; *Schefflera arboricola*; *Panax ginseng*; cis-9,17-octadecadiene-12,14-diyne-1,16-diol; falcarinol; plants; allergic contact dermatitis. © Munksgaard, 1999.

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In our previous report (1), we found cis-9,17-octadecadiene-12,14-diyne-1,16-diol (**I**) to be 1 of the major allergens of *Dendropanax trifidus*. Although we suspected there were other allergens contained in *Dendropanax trifidus*, we were unable to elucidate the allergens. In that same report, 4 of 6 patients who had been sensitized to *Dendropanax trifidus* showed reaction to *Fatsia japonica* (Fig. 1), which is also a member of the Araliaceae family. These results suggest that the same or cross-reacting allergens were contained in the these 2 plants (2–5).

Contact dermatitis due to *Hedera helix* (6–10), *Hedera canariensis* (8), *Schefflera arboricola* (11), and *Brassia actinophylla* (syn. *Schefflera actinophylla*) (6), all of which belong to the Araliaceae family, has been reported, and the major allergen of these plants demonstrated to be falcarinol (7, 8, 11). Cross-reaction between these plants and *Fatsia japonica* has been discussed (6, 10), although *Fatsia*

japonica was not found to contain falcarinol (12). In our earlier report (1), 1 patient with hypersensitivity to the leaves of *Dendropanax trifidus* and *Fatsia japonica* showed a positive reaction to the leaf of *Hedera helix* on patch testing. This again suggests cross-reaction between the plants. The purpose of this study was to: (i) evaluate the other allergens in *Dendropanax trifidus*, (ii) identify the allergens in *Fatsia japonica*, and (iii) investigate the cross-sensitivity of these allergens with other plants in the Araliaceae family.

Materials, Methods and Results

Subjects

All subjects from our previous study, with the exception of case nos. 6 and 7, participated in this study. Case nos. 1 to 5 are the same subjects as case nos. 1 to 5 in our previous report (1).

Case no. 1 was a 75-year-old man with a pre-



Fig. 1. *Fatsia japonica* Decne. et Planch.

vious history of contact dermatitis due to *Dendropanax trifidus*, *Toxicodendron verniciflua* and *Primula obconica*, and case no. 2 was a 43-year-old woman with a previous history of acute dermatitis after pruning the leaves of *Dendropanax trifidus*. On patch testing, both patients had shown positive reactions to the leaves of *Dendropanax trifidus* and *Fatsia japonica* and compound I.

Case nos. 3–5 were males, aged between 29–30 years. They had participated in previous patch testing as healthy control subjects, but in the course of the experiment were actively sensitized by the leaf of *Dendropanax trifidus* and compound I. Case no. 3 had also shown a flare-up to the leaf of *Fatsia japonica*.

Patch testing

Test samples were applied on the flexor side of the upper arm, using Finn Chambers® (Epitest Ltd., Helsinki, Finland) and Scanpor® (Norgesplaster A/S, Oslo, Norway) tape. Reactions were read at 2, 3 and 7 days according to the ICDRG classification.

(A) Evaluation of the other allergens in *Dendropanax trifidus*

Isolation of allergens from *Dendropanax trifidus*

The plant material was obtained from one of the authors' garden. The method of isolation of aller-

gens from fresh leaves of *Dendropanax trifidus* was described in our previous report (1). The HPLC pattern of the ethanol saturated resinous sap solution was very similar to that of 70% methanol solution of leaves. Some further experiments were performed using ethanol-saturated resinous sap. The sap was roughly separated into 29 fractions by ODS column chromatography. The fractions were diluted in white petrolatum for patch testing.

Patch testing with fractions and their results

The 29 fractions were patch tested on 2 patients (case nos. 1 and 2). Both patients reacted positively to 2 of the fractions.

Purification and analysis of allergens

The 2 fractions responsible for the above positive reactions were purified 3× under several conditions by HPLC, using an ODS column, and preserved in argon to prevent polymerization. 3 compounds suspected as allergens were obtained (Fig. 2). 2 compounds were purified from the higher polar fraction. These were *cis*-9,17-octadecadiene-12,14-diyne-1,16-diol (I), which was reported previously, and 16-hydroxy-*cis*-9,17-octadecadiene-12,14-diyne-1,16-diol (II). *cis*-9,trans-16-Octadecadiene-12,14-diyne-1,16-diol (III) was purified from the lower polar fraction. They were identified by ¹H NMR and ¹³C NMR analyses and HR mass spectrometry (13). Estimated yields of each compound (I–III) in 1 ml ethanol saturation resinous sap were 4.0 mg, 0.84 mg, and 3.4 mg, respectively.

Evaluation of the allergenicity of compounds I, II and III

5 subjects (case nos. 1–5) participated in this study. They were patch tested with the compounds and

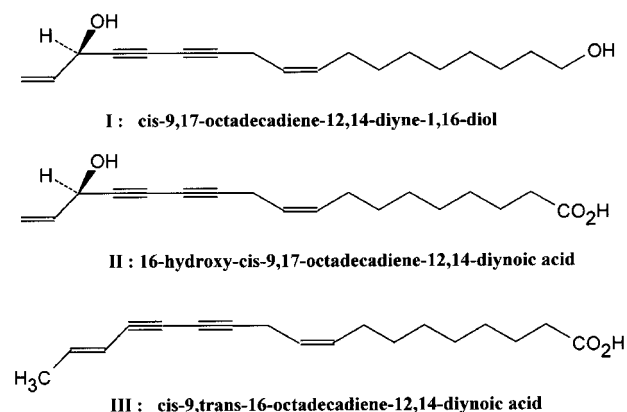


Fig. 2. Structure of allergens in *Dendropanax trifidus* Makino.

Table 1. Results of patch testing with 3 compounds in *Dendropanax trifidus*

	conc. (%)	Case no.				
		1	2	3	4	5
I	0.05	++	++	++	+	+
	0.01	++	++	++	+	+
	0.02	+	++	+	—	—
II	0.05	++	++	++	+	—
	0.01	++	++	++	+	—
	0.02	+	++	+	—	—
III	0.05	—	++	+	+	—
	0.01	—	++	+	—	—
	0.02	—	+	—	—	—

I; cis-9,17-octadecadiene-12,14-diyne-1,16-diol.

II; 16-hydroxy-cis-9,17-octadecadiene-12,14-diyneic acid.

III; cis-9,trans-16-octadecadiene-12,14-diyneic acid.

Each compound was diluted with petrolatum.

all reacted positively (Table 1). Compounds **I** and **II** elicited similarly strong reactions in low concentrations. Compound **III** also elicited reactions in 3 subjects (case nos. 2–4) but the reactions were weaker.

(B) Identification of the allergens in *Fatsia japonica*

Isolation of allergens from *Fatsia japonica*

31.6 g of fresh leaves of *Fatsia japonica* were soaked in 300 ml of methanol for 1 day at room temperature and filtered. The filtrate was evaporated to dryness and dissolved in CHCl_3 , dried (Na_2SO_4), and concentrated. The residue was suspended in 70% methanol (50 ml) and centrifuged for 30 min (10,000 rpm). The supernatant was concentrated and dissolved in 50% ethanol (10 ml) and then chromatographed for pretreatment (column; TOYOPAK ODS, solvent; CH_3CN 10 ml). The eluates were concentrated and suspended in 5 ml of 60% CH_3CN - H_2O containing 0.1% of CF_3COOH (0.1% TFA) and centrifuged (3,500 rpm, 5 min). The supernatant was roughly separated into 24 fractions by ODS column chromatography, with a 30 min linear gradient from 60% to 100% acetonitrile in 0.1% TFA solution, and then concentrated under reduced pressure.

Patch testing with fractions and their results

2 subjects, cases no. 1 and 2, were patched with the 24 fractions diluted in white petrolatum. Both patients reacted positively to 1 fraction.

Purification and analysis of allergens

The active fraction was suspended in 55% CH_3CN -0.1% TFA and centrifuged. The supernatant was

Table 2. Results of patch testing in patients with hypersensitivity to *Dendropanax trifidus* with the leaves or extracts of Araliaceae family plants

Case no.	1	2	3	4	5
<i>Hedera helix</i>	++	++**	++	++	+
<i>Schefflera arboricola</i>	++	++	++	++	++
<i>Panax ginseng</i> *	++	—	—	—	—

*: The ethanol extract of the purchased *Panax ginseng* root powder (1%).

**: Case no. 2 showed no reaction when patch tested with the leaf of *Hedera helix* the 1st time, but showed positivity when patch tested a 2nd time.

subjected to analytical HPLC and 7 compounds obtained. The compounds were diluted in white petrolatum and patch tested on the same 2 subjects.

Both subjects showed a positive reaction to 1 of the compounds. Analysis of this active compound was performed with ^1H -NMR, ^{13}C -NMR, HRMS, UV, IR. The compound was found to be compound **I**, previously reported by us as a major allergen of *Dendropanax trifidus*. 0.23 mg of compound **I** was obtained from 31.6 g of fresh leaves of *Fatsia japonica*.

(C) Investigation into the cross-sensitivity of compound **I** with other plants in the Araliaceae family

5 subjects (case nos. 1–5) were patch tested with the whole leaves of *Hedera helix* and *Schefflera arboricola*, and the ethanol extract of the purchased *Panax ginseng* root powder (1%, Sigma). 4 subjects showed positive reactions to the leaves of *Hedera helix* and *Schefflera arboricola*. 1 subject (case no. 2) showed no reaction to the leaf of *Hedera helix* the 1st time, but reacted positively when patch tested a 2nd time. She also showed a positive reaction to the leaf of *Schefflera arboricola*. 1 out of 5 subjects (case no. 1) showed a positive reaction to the ethanol extract of *Panax ginseng* root (Table 2).

10 healthy subjects aged between 24 and 38 years, with no history of contact dermatitis due to these plants, were patch tested with the leaves of *Hedera helix* and *Schefflera arboricola* and the ethanol extract of *Panax ginseng* root. 1 subject showed a positive reaction to the leaf of *Schefflera arboricola*. She was then patch tested with the leaves of *Dendropanax trifidus* and *Fatsia japonica*, and compound **I** diluted to 0.05% with petrolatum, 2 weeks later. She reacted positively to the leaves of both plants and compound **I**. The other subjects did not show any reaction.

Discussion

Dendropanax trifidus and *Fatsia japonica*, belonging to the family Araliaceae, are ubiquitous evergreen plants found in the west of Japan and commonly cultivated in gardens for their handsome foliage. The stem of *Dendropanax trifidus* is erect and the leaves are 3-lobed and glossy green in color (14). Contact dermatitis due to *Dendropanax trifidus* is reported only from Japan these days, although the resinous sap of the plant was used as a dark yellow varnish in the 8th century in both Japan and Korea (15). *Fatsia japonica* (16) is an erect shrub with few branches and can grow to a height of 2.5 m. Its large leaves are rich and glossy in color. They are palmate, with 7 to 9 coarsely toothed oblong-lanceolate lobes (Fig. 1). *Fatsia japonica* was introduced from Nagasaki, Japan, to Europe by a German named Dr. Siebold, who was then an employee of the East India company.

In our previous report (1), compound **I** was confirmed to be the major allergen of *Dendropanax trifidus* and a strong sensitizer. The amount of compound **I** was abundant and did not vary among samples. Other constituents of the fractions which also elicited reactions on the 2 patients with sensitivity to *Dendropanax trifidus* were considered to contain analogues of compound **I**. However, we were unable to identify the constituents because of the difficulty in purifying the fractions, due to lack of quantity of each sample, which were also unstable. Thus the fractions were stored in argon to prevent polymerization and decomposition, which led to success in identifying compound **II** and **III** as the constituents of the fraction. They belong to the polyacetylenes. Compounds **II** as well as **I** were first purified and analyzed from *Dendropanax trifidus* in 1973 (17), and they were reported as antifungal agents. Compound **III** was purified as a varnish constituent (15) just like compounds **I** and **II** in *Dendropanax trifidus*.

Our previous study demonstrated 2 patients with contact dermatitis due to *Dendropanax trifidus* with positive responses to the leaf of *Fatsia japonica*. 2 of 4 control subjects sensitized to *Dendropanax trifidus* and compound **I** showed flare-ups when patch tested with the leaf of *Fatsia japonica*. The results of our previous paper also supported previous findings (2–5) that the same or cross-reacting agents are found in the 2 plants. In this report, the allergen of *Fatsia japonica*, which is also compound **I**, was first evaluated. It was the same compound as the major allergen of *Dendropanax trifidus*, but its concentration in *Fatsia japonica* was 7× less than in *Dendropanax trifidus*. This could be the reason why case reports of contact dermatitis due to *Fatsia japonica* are so few,

whereas cases of contact dermatitis due to *Dendropanax trifidus* are more commonly reported (1–5, 18, 19).

Hedera helix L., *Hedera canariensis*, *Schefflera arboricola*, and *Brassaia actinophylla* are all plants of the Araliaceae family reported to cause contact dermatitis (6–11). Falcarinol, first isolated from *Panax ginseng* C. A. Meyer (20), was isolated from *Schefflera arboricola* and identified as the major allergen of the plant (11). Falcarinol (7, 8) was also identified from *Hedera helix* and found to act as a major allergen. Didehydrofalcarinol and 11,12-dehydrofalcarinol were also elucidated from *Hedera helix* as allergens. Mitchell (6) reported a case of contact dermatitis from *Hedera helix* and *Brassaia actinophylla* with a positive patch test reaction to the leaf of *Fatsia japonica*. García et al. (10) reported a case of contact dermatitis due to *Hedera helix* and suggested that the positive reaction to the stem of *Fatsia japonica* (a hybrid of *Hedera helix* and *Fatsia japonica*) on patch testing was due to sensitivity to both plants.

However, falcarinol was reported to be absent in *Fatsia japonica* (12), and the active components of *Dendropanax trifidus* and *Fatsia japonica* elucidated in this study were compounds **I**, **II**, and **III**. We found that 5 patients with hypersensitivity to the leaves of *Dendropanax trifidus* and compounds **I**, **II**, and **III** showed positive reactions to the leaves of *Hedera helix* and *Schefflera arboricola*. They did not have any past history of contact with these plants. 1 healthy subject who showed a positive reaction to the leaf of *Schefflera arboricola* also showed positive reactions to the leaves of *Dendropanax trifidus* and *Fatsia japonica* and compound **I**. She had a history of contact with *Dendropanax trifidus*, but not with *Schefflera arboricola*. We concluded that there are cross-reactions among these plants.

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