Arch Dermatol Res (1989) 281:227–230

Archives of Dermatological Research

Allergic contact dermatitis to *Ginkgo biloba* L.: relationship with urushiol

J.-P. Lepoittevin[1, 2], C. Benezra[1], and Y. Asakawa[2]

[1] Laboratoire de Dermatochimie associé au CNRS (UA 31), Université Louis Pasteur, Clinique Dermatologique, CHU, F-67091 Strasbourg, France
[2] Institute of Pharmacognosy, Tokushima Bunri University, Yamashiro-Cho, Tokushima 770, Japan

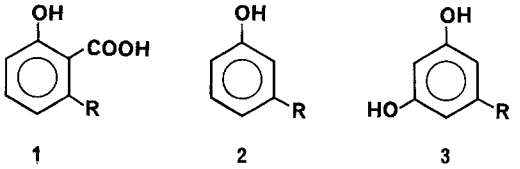
Summary. A *Ginkgo biloba* L. fruit extract was prepared and purified. Three groups of guinea pigs were sensitized to the crude extract, anacardic acids 1, and cardanols 2 respectively, using the FCAT method, and the fourth group to urushiol using the epicutaneous route. Each group was tested for reaction to the primary sensitizer and to the different main aromatic compounds isolated from Ginkgo fruits. Anacardic acids were found to be good sensitizers, while cardanols failed to induce allergic contact dermatitis (ACD). No cross-reactions were observed among the compounds tested. Ginkgolic acids 1 seem to be the main allergens of *Ginkgo biloba* L. and the hypothesis of a biotransformation of 1 into catechol 4 is not supported by experiment.

Key words: *Ginkgo biloba* L. – Delayed hypersensitivity – Urushiol – Anacardic acids – Cardanols – Cardols

In our study of allergic contact dermatitis (ACD) to natural long-chain alkyl compounds we were interested in ACD to *Ginkgo biloba* L. and its related reactions with urushiol.

Ginkgo tree (*Ginkgo biloba* L.), the sole modern surviving species of the group of plants known as *Ginkgoales*, whose ancestry has been traced to more than 200 million years, has been known for a long time as a sensitizing plant [5]. For example, in the autumn of 1963, there was an epidemic of ACD among the students of a preparatory school for girls due to the fallen fruits of a female Ginkgo tree: 35 cases were observed [7]. More recently, 3 cases were reported in Strasbourg in November 1987 [9]. Cross-reactivity between the ginkgo fruit pulp and poison ivy was demonstrated [7]. Furthermore, ginkgo fruit, Japanese lacquer, cashew nut shell oil, and mango rind have been reported to react with the poison ivy-oak-sumac group [1].

The main aromatic components of ginkgo fruits have been identified [3] as anacardic acids 1, cardanols 2, and cardols 3. Each group consists of a complex mixture of homologues with alk(en)yl chains containing from 13 to 19 carbons and from 0 to 2 double bonds (see Fig. 1). The ginkgolic acids 1 (the main aromatic compounds) were suspected to be the allergens of ginkgo (results based on challenge tests), but no systematic study on the allergenicity of the aromatic components of ginkgo has been made and the nature of the allergen is still a subject to speculation. The close structure between ginkgolic acids 1 and components of urushiol 4 (the allergen of poison ivy), and cross-reactions reported in the literature, led some authors [2, 4] to suggest that alkylcatechols, resulting from an in vivo biotransformation of 1 in the skin could be the "actual" allergen of *Ginkgo biloba* L. (Fig. 2).

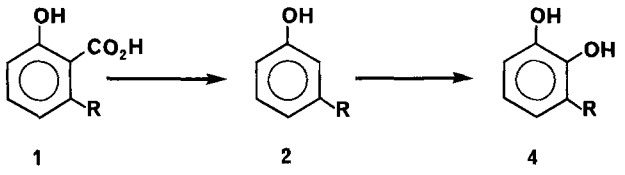


1 2 3

R = $C_{13}H_{27}$
= $C_{15}H_{31}$, $C_{15}H_{29}$
= $C_{17}H_{35}$, $C_{17}H_{33}$, $C_{17}H_{31}$
= $C_{19}H_{37}$

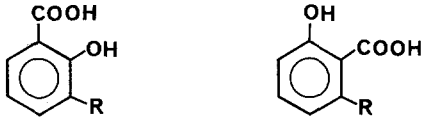
Fig. 1. Main aromatic compounds from *Ginkgo biloba* L.: tridecyl-, pentadecyl-, pentadecenyl-, heptadecyl-, heptadecenyl-, heptadecanienyl-, and nonadecenyl-salicylic acids 1, cardanols 2 and cardols 3

Offprint requests to: Jean-Pierre Lepoittevin (address see above)



1 2 4

Fig. 2. Possible biotransformation of anacardic acids. Anacardic acids 1 (see above) could be decarboxylated to yield cardanols 2 and oxidized into pyrocatechols 4
R = $C_{15}H_{31}$



5 6

Fig. 3. Synthetic analogs of anacardic acids: 3-pentadecyl (5) and 6-pentadecyl (6) salicylic acids

If the first step occurs spontaneously in solution (1 has been demonstrated to be the precursor of 2 in the plant), the direct oxidation of 2 to 4 which has recently been achieved chemically (J.-P. Jepoittevin and Y. Asakawa, unpublished results, 1986), is still the subject of speculation as an in vivo process.

In an attempt to elucidate the true structure of ginkgo allergen(s) and test the hypothesis of an in vivo biotransformation of 1, we now report the first animal sensitization studies on *Ginkgo biloba* L. components.

Material and methods

Chemicals

The crude extract of *Ginkgo biloba* L. fruits, collected in the Tokushima area (Japan) in the autumn of 1984, was prepared as previously reported [3]. The extract was chromatographed on silica gel using the "flash" chromatography technique [8] and the eluent was a mixture of *n*-hexane, ethyl-acetate, and acetic acid (90:10:1). The crude fractions were then purified by chromatography on Sephadex LH 20 using chloroform-methanol (1:1) as eluent. Urushiol (extracted from poison ivy) was kindly provided by Prof. G. Dupuis, Sherbrooke University. 3-Pentadecylsalicylic acid 5, saturated analog of ginkgolic acids, and 6-pentadecylsalicylic acid 6, isomer position of 5, have been synthesized in our laboratory by D. Huber (unpublished results).

Animal sensitization

Female Himalayan spotted guinea pigs (Füllingsdorf, Switzerland), weighing 250–350 g, were used. Sensitization induction treatment to the crude extract, to 1 and 2 product was achieved by Freund's complete adjuvant test (FCAT); groups of eight animals were used. Each animal received three intradermal injections (0.1 ml emulsion) into the nuchal region on alternate days. The experimental groups received emulsion made up of the sensitizing substances in a 1:1 FCA-saline mixture at the concentrations reported in Table 1. The control group received injection of 1:1 FCA-saline emulsion only. Guinea pigs were sensitized to urushiol 4 epicutaneously: 1 mg (3.0 μmol) of urushiol in 100 μl of acetone:olive oil (4:1) was applied to a 4-cm^2 area of the clipped and shaved flank of the guinea pigs.

Table 1. Sensitization[a] doses

Compound	(%)	(μmol)
Anacardic acid 1	1	0.7
Crude extract	1	–
Cardanol 2	1	0.8

[a] The animals were sensitized by the FCAT method using three injections of the above compounds in a 1:1 FCA/saline mixture

Skin testing

After a period of 21 days after the beginning of sensitization, the animals were challenged by depositing 25 μl of a solution (acetone) of the substance to be tested on a circular 2-cm^2 area of the clipped and shaved flank (at a nonirritating concentration).

Skin reactions were read 24, 48, and 72 h after application of the challenge dose and were rated according to the following scale: 0, no reaction; 0.5, discrete erythema; 1, confluent erythema; 2, erythema with infiltration and edema; 3, erythema extending well beyond the testing area; 4, ulceration or necrosis.

For purposes of comparison, a numerical average response value was calculated for each set of readings by summing up the individual rating and dividing the sum by the total number of animals in the experimental group.

Results

Assessment of primary irritating threshold dose

Prior to initiating the study, we treated a group of naive animals with varying amounts of the products to be tested (0.08 to 8.2 μmol/2 cm^2) to determine the primary irritating threshold dose (Table 2) and the appropriate dose range to assess induction of hypersensitivity. Readings at 24, 48, and 72 h after testing established that cardanols exhibited cutaneous toxicity at the 0.2 μmol level whereas the other materials, anacardic acids and the crude extract, showed little or no toxicity. Cardanols in the crude extract have to be prepared immediately before testing, decarboxylation of 1 leading to the more toxic compound 2.

Sensitization capacity

Table 3 shows the results of skin testing on different animal groups. Guinea pigs sensitized with the crude extract, ginkgolic acids, and urushiol reacted with the primary sensitizer with a significant intensity (average 1.0 reaction). The maximum intensity of the skin reaction was reached 48–72 h after the epicutaneous challenge, a response characteristic of allergenic long-

Table 2
Primary toxicity test to the main aromatic compounds isolated from *Ginkgo biloba* L.

Compound	(%)	(μmol)	Animals with a test[a] intensity of (*n*)				Average skin response	Sensitive/ total
			2	1	0.5	0		
Crude extract	10	–	0	1	3	0	0.6	4/4
	3	–	0	0	0	4	0	0/4
	1	–	0	0	0	4	0	0/4
Anacardic acids	10	7.2	0	0	0	4	0	0/4
	3	2.2	0	0	0	4	0	0/4
Cardanols	10	8.3	0	0	4	0	0.5	4/4
	3	2.4	3	1	0	0	1.7	4/4
	1	0.8	2	2	0	0	1.5	4/4
	0.3	0.2	0	0	1	3	0.1	1/4
	0.1	0.1	0	0	0	4	0	0/4

[a] The animals were challenged by depositing 25 μl of an acetone solution on the shaved flank of the animal (on a 2-cm^2 circular area)

Table 3. Results of open epicutaneous test

Compound	(%)	(μmol)	Animals with a test intensity of (*n*)[a]				Average skin response	Sensitive/ total
			2	1	0.5	0		
Group I: Sensitized with the crude extract								
Crude extract	3	–	1	6	1	0	1.1	8/8
Anacardic acids 1	10	7.2	5	3	0	4	1.6	8/8
Cardanols 2	0.3	0.2	0	0	4	4	0.25	4/8
Cardols 3	0.1	0.1	0	0	0	8	0	0/8
Urushiol 4	0.05	0.04	0	0	0	8	0	0/8
Compound 5	2	1.4	0	0	0	8	0	0/8
Compound 6	2	1.4	0	7	1	0	1	8/8
Group II: Sensitized with anacardic acids 1								
Crude extract	3	–	0	3	5	0	0.7	8/8
Anacardic acids 1	10	7.2	1	5	2	0	1.0	8/8
Cardanols 2	0.3	0.2	0	0	0	8	0	0/8
Cardols 3	0.1	0.1	0	0	0	8	0	0/8
Urushiol 4	0.05	0.04	0	0	0	8	0	0/8
Compound 5	2	1.4	0	0	0	8	0	0/8
Compound 6	2	1.4	0	4	4	0	0.75	8/8
Group III: Sensitized with cardanols 2								
Crude extract	3	–	0	0	0	8	0	0/8
Anacardic acids 1	10	7.2	0	0	0	8	0	0/8
Cardanols 2	0.3	0.2	0	0	0	8	0	0/8
Cardols 3	0.1	0.1	0	0	0	8	0	0/8
Urushiol 4	0.05	0.04	0	0	0	8	0	0/8
Group IV: Sensitized with urushiol 4								
Crude extract	3	–	0	0	0	8	0	0/8
Anacardic acids 1	10	7.2	0	0	0	8	0	0/8
Cardanols 2	0.3	0.2	0	1	5	2	0.4	6/8
Cardols 3	0.1	0.1	0	0	0	8	0	0/8
Urushiol 4	0.05	0.04	0	7	1	0	1	8/8
Group V: Control group showed negative reactions to all tested substances								

[a] Elicitation was performed by depositing 25 μl of an acetone solution on the shaved flank of the animal (on a 2-cm^2 circular area) and tests were read at the 48th h using a 0 (no reaction) to 3 (strong reaction with swelling going beyond the test area); average skin reactions calculated by adding the numbers (0–3) and dividing by the number of tested animals

chain compounds. The group treated with cardanols showed no allergic reaction when challenged.

Cross-reactions

Except for the one observed between anacardic acids and crude extract, no clear cross-reactions could be established. We noticed a slight reaction to cardanols 2 in the group sensitized to urushiol. Cardanols, which in our study were not sensitizers, seem to be able to behave as elicitors.

Discussion

It seems obvious from the above results that ginkgolic acids 1 are the allergens of *Ginkgo biloba* L. and that the hypothesis of a biotransformation of 1 into catechols 4 is not supported by the experiment.

The crude extract of *Gingko biloba* L. fruits is a sensitizer and the only aromatic compounds to cross-react with it are anacardic acids. Furthermore, this study confirms that 1 is a sensitizer even if it seems to be a little less potent than the crude extract. Compounds 3, which are suspected to be the allergens of *Philodendron* species [6] seem to be present in amounts too small to play any part in ACD to ginkgo fruits.

The more surprising result of this study was the lack of cross-reaction between Ginkgo and urushiol, a cross-reaction which is often reported in the literature. Based on this evidence, the hypothesis of a biotransformation of anacardic acids into catechols via cardols can no longer be supported. In addition, cardanols, which could have been the precursors of the catechols were found to be inactive.

The results reported in the literature can be interpreted in the following way. First, the cross-reactions reported are probably true polysensitization, patients being in contact with both urushiol and ginkgo; second, the reactions observed are irritating responses. It is well known that urushiol is toxic at very low concentrations and that anacardic acids give spontaneous decarboxylation reactions to produce much more toxic cardanols. Finally, metabolism in the human skin may be different from that in guinea pig skin.

We have also tested two synthetic alkylsalicylic acids (D. Huber and C. Benezra, unpublished results), 6- and 3-pentadecyl salicylic acid (compound 5 and 6, see Fig. 3), in order to observe the specificity of ACD to such compounds versus the position of the side chain. While 5, which is a saturated analog of 1, gave a positive reaction, 6 showed no activity as an elicitor on guinea pigs sensitized to the crude or anacardic acids.

In conclusion, this study stresses the great care which must be exercised when talking of cross-reactions, especially, in patients whose allergenic past is unknown.

Acknowledgement. We thank the Ministère des Relations Extérieures (France) for the financial support to JPL during his stay in Japan.

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Received June 3, 1988