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ALLERGIC CONTACT DERMATITIS FROM *FRULLANIA* AND COMPOSITAE

THE ROLE OF SESQUITERPENE LACTONES*

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ABSTRACT

Fifteen forest-workers with allergic eczematous contact dermatitis were found to be allergic to *Frullania*, a genus of liverworts. Five of these patients, so tested, also showed positive patch test reactions to Compositae species. Sesquiterpene lactones were shown to be the common denominators of such dermatitis. The patients reacted to a number of these compounds that differed in their skeleton and functional groups. The most active compound found was alantolactone. The immunochemical requisite of the molecular structure of these compounds appeared to be the presence of a carbon-carbon double bond conjugated with the lactone group.

In an investigation of some sensitizing agents for workers in the forest industries of British Columbia, we have observed 15 cases of allergic contact dermatitis from *Frullania,* a genus of liverworts. Seven of these cases have been reported (1). In five patients so tested, positive patch test reactions have also been obtained from some species of the tribes Heliantheae and Anthemidae of the Compositae family. Species in these and other tribes of the Compositae have been reported to cause allergic contact dermatitis (2).

Investigation of *Frullania nisqualensis* for its allergenic fractions indicates that sesquiterpene lactones are probably the common denominators of allergic contact sensitization by *Frullania* and by some species of Compositae.

We wish to thank Dr. W. A. McLeod and Dr. W. D. Stewart who referred their patients for investigation and the Photographic Department, Shaughnessy Hospital, Vancouver 9, British Columbia for the photograph in Figure 2. Dr. A. K. Roy kindly carried out patch tests in his patients. Professor Ourisson and Professor Geissman, in addition to providing chemicals for patch testing, generously gave advice and suggestions. Dr. W. B. Schofield and Dr. V. J. Krajina, Department of Botany, The University of British Columbia generously gave their time towards identification of *Frullania*. Patients who submitted to very numerous instances of patch test dermatitis have our special thanks.

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MATERIALS AND METHODS

1. *Patch-testing.* Portions of the plant body of *Frullania nisquallensis* (Fig. 1) were used for patch testing. Patches were removed at 24 hours; reactions were read at 24, 48 hours, 5 and 14 days and scored by the method of Witten and Shair (3).

Plant oleoresins were obtained from Hollister-Steir Laboratories, Spokane, Wash. Twenty-four hour closed patch tests were carried out on the skin of the back or outer upper arm using patch test plasters No. 71, Duke Laboratories Inc., South Norwalk, Conn.

Patch testing with chemicals was carried out with 80% aqueous ethyl alcohol solutions, unless otherwise indicated, using approximately 30 μl of the solution. The chemicals tested were without primary irritant effect in the concentrations used.

Some other species of *Frullania* were donated by Dr. P. L. Redfearn, Southwest Missouri State College, Springfield, Mo.

2. *Chemicals.* Arbusculin B (II, Table II), (IV), arbusculin A (V), cumambrin B (XI), cumambrin A (XII), formylcumambrin B (XIII), tetrahydrocumambrin B (XIV), dihydrocumambrin B (XV), coronopilin (XVI) and ambrosin (XVII) were kindly donated by Professor T. A. Geissman, Department of Chemistry, University of California at Los Angeles. A sample of Compound I (Table II) was kindly donated by Professor G. Ourisson, Department of Chemistry, University of Strasbourg. Santonin (VI) and a mixture of alantolactone

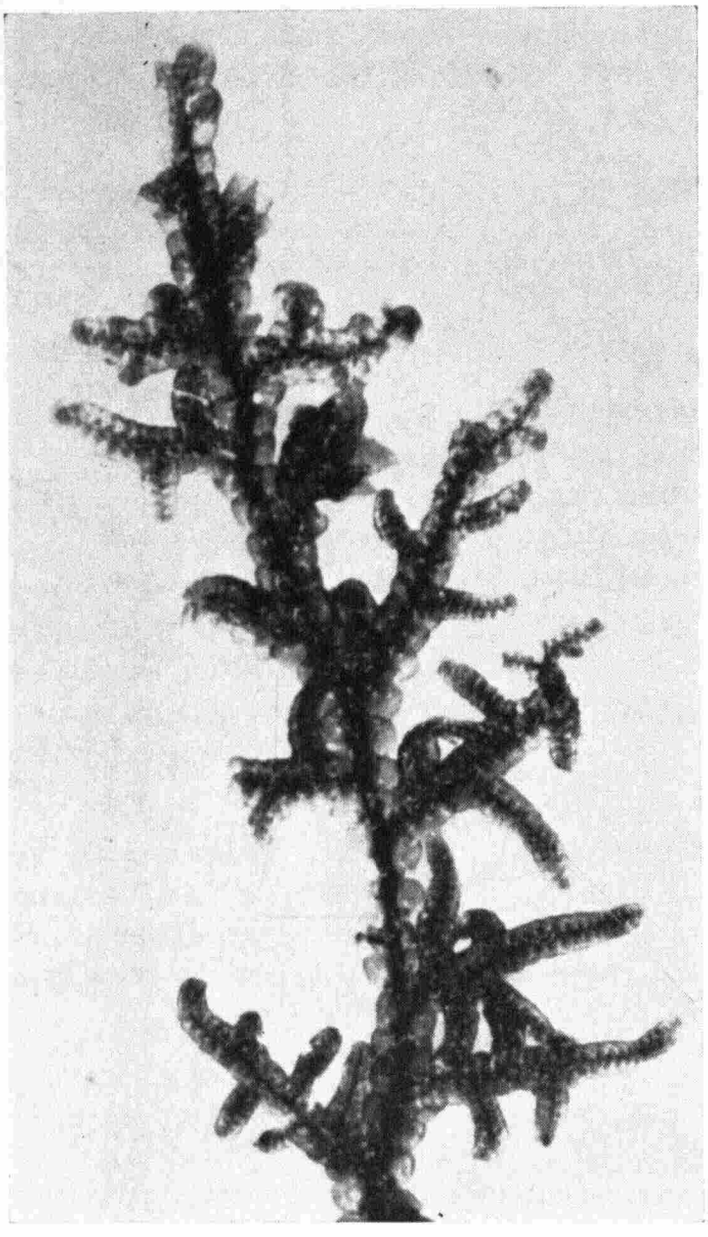

FIG. 1. *Frullania nisquallensis*. × 190.

(VII) and isoalantolactone (VIII) (commercial name, helenin) were obtained from Sigma Chemical Co., St. Louis, Mo. To separate the last two lactones, a mixture was run on a column of silica-gel containing 10% of $AgNO_3$ with ether-petroleum ether (15–85) as eluant. Each substance was then recrystallized from petroleum ether.

Tetrahydroalantolactone (IX) was obtained from alantolactone (VII) by catalytic hydrogenation. Dihydroalantolactone (X) and dihydroarbusculin B (III) were prepared respectively from alantolactone (VII) and arbusculin B (II) by reduction with $NaBH_4$ in ethyl acetate.

3. *Isolation of allergens from* Frullania nisquallensis. 500 gm dry weight of *Frullania nisquallensis* were collected in forest areas of British Columbia. The plant material was ground up and extracted several times with ethyl ether. Chromatography of the ether extract was carried out with silica-gel as adsorbent. Several runs with various concentrations of ether in petroleum ether as eluant were performed and final purification was achieved by fractional sublimation. Two active substances were isolated. Their behaviour after thin-layer chromatography (TLC) with ethyl acetate-cyclohexane (10/90) as eluant has been compared with that of arbusculin B and alantolactone and is as follows: Not yet identified: $R_f = 0.32$; Compound I: $R_f = 0.25$; Arbusculin B (II): $R_f = 0.37$; Alantolactone (VII): $R_f = 0.22$. When the TLC plates were sprayed with a mixture of H_2O-H_2SO_4 (50/50) and then heated at 200° C for 2 minutes, a deep blue colour appeared at the level of compounds I and II which are diastereoisomers. This is a sensitive test for the detection of these two compounds.

CASE REPORTS

(1) A white male retired electrician and active gardener, aged 77 years, had recurrent dermatitis for 40 years. Fifteen years previously, persistent dermatitis appeared. The eruption was confined to the exposed skin surfaces except during the summers when it became partially generalized. Six years previously, he was found allergic to chrysanthemums and he subsequently avoided contact with these plants. Strong positive patch test responses were observed following application to the skin of *Frullania* plant (as is) and extracts of chrysanthemum. Both clinically and by photo-testing, there was evidence of photosensitivity and he was also an atopic individual. (A fuller report of this patient has been presented in Ref. 4.)

(2) A white male retired air-craft fitter and active gardener, aged 82 years, showed dermatitis of the exposed skin surfaces. Admission to hospital was required yearly during the summer and early fall from 1957–1967. Five years previously he was found allergic to chrysanthemums and he subsequently avoided contact with these plants. During the winter, the dermatitis improved markedly but did not entirely clear. Clinically and by photo-testing there was evidence of photosensitivity.

(3) A white male logger, aged 55 years, had noted attacks of dermatitis affecting the hands and forearms during work in forest areas for 15 years. The dermatitis appeared within two days of starting work, was more severe in wet weather, and cleared during three weeks after leaving work. He was unaffected while at home. There was no clinical evidence of photosensitivity. He had no known contact with chrysanthemums.

(4) A white male logger, aged 64 years, had recurrent dermatitis of the exposed skin surfaces for 45 years. For 40 years, the dermatitis occurred only during work in forest areas; in the five years after he retired from the forest industry, dermatitis appeared during July to October when he was at home. He became a chrysanthemum fancier after retirement and was of the opinion that he was allergic to some plant in the woods which he could not identify, and to chrysanthemums. This was correct, as it turned out. Patch test reactions to certain plants in his case are shown in Fig. 2.

(5) A white male physician, aged 46 years, became sensitized to *Frullania* in the fall of 1968 while patch testing himself with some extracts of this plant. There has been no significant evidence, to date of clinical dermatitis or of photosensitivity.

RESULTS

No primary irritation resulted from application of the *Frullania* plant to the skin of 30 apparently normal individuals. In the five cases described here, positive patch test reactions were observed following application of the plant for 24 hours. It was not necessary to 'bruise' or injure the plant body. Positive reactions were observed on removal of the patches at 24 hours. Such reactions reached a height of intensity in 1 to 5 days and persisted for up to 30 days, occasionally for as long as two months. In addition, a localized urticarial reaction usually appeared in Case 1, occasionally in Case 4, and lasted a day. In earlier tests, we applied a larger sample of the plant and observed phenomenon of a "pressure patch test" described by Epstein and Kligman (5). The application of several layers of the plant body under tape produced a slight indentation of the skin. On removal of the patch at 24 hours, only this indentation and some local erythema were visible. Within an hour, the erythema became brighter and oedema of the skin appeared; exudation appeared later. The intensity of dermatitis produced by patch test challenge with *Frullania* plant was comparable with the highest that we have observed with any chemical. A regular feature of the dermatitis was that it became more intense during the few days following removal of the patch at 24 hours and that, when it occurred, exudation was copious. In Cases 1 and 4, a flare-up of clinical dermatitis occurred after patch testing.

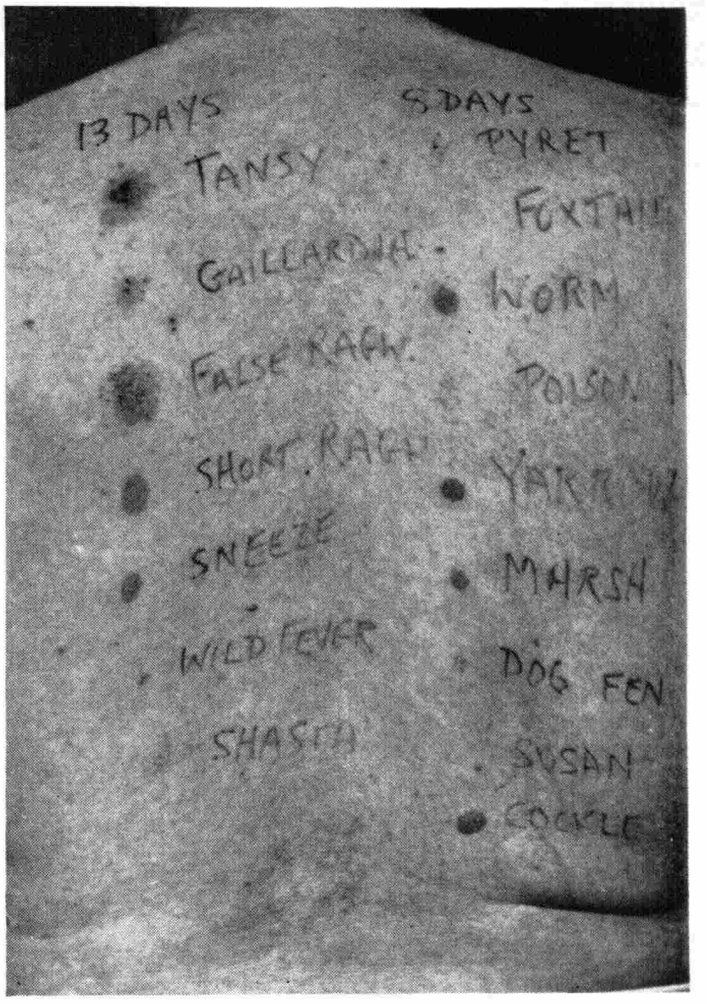

FIG. 2. Patch test reactions in Case 2, 13 days after removal of patches: tansy, gaillardia, shasta daisy 1/10 v/v; false ragweed, short ragweed, sneezeweed, wild feverfew 1/20 v/v, in acetone. 8 days after removal of patches: pyrethrum 10% in petrolatum; foxtail grass, wormwood, yarrow, marshelder, dog fennel, black-eyed susan, cocklebur 1/10 v/v in acetone; poison ivy 1/50 v/v in acetone. Readings made at 48 hours viz. 24 hours after removal of the patches are scored in Table I.

Some observations were made on the duration of application of the plant required to produce a dermatitis. In three other patients, not otherwise reported here, *Frullania* plant was applied to the skin under Blenderm® surgical tape. When a minimal erythema appeared, the tape was removed together with the plant. The minimal erythema time averaged 80 minutes in one patient, somewhat longer in the other two. Dermatitis appeared over night at the test sites, usually with exudation, and the patch dermatitis persisted for several weeks.

TABLE I

Patch test results in Cases 1–5. (48 hours readings scored by the method of Witten and Shair (3))

Botanical name	Common name	Vehicle	%v/v	Patch test results Patient				
				1	2	3	4	5
Family: Compositae								
Tribe: Anthemidae								
Achillae lanulosa	Yarrow	acetone	10	4	0	0	3	2
Anthemis cotula	Dog Fennel	acetone	10	3	3	0	1	0
Artemisia absinthium	Wormwood	acetone	10	0	2	0	2	0
Chrysanthemum spp.	Wild feverfew	acetone	5	0	2	0	4	0
C. coccineum	Pyrethrum	petrolatum	10	3	0	0	0	0
C. maximum	Shasta daisy	acetone	10	0	0	0	0	0
C. parthenium	Feverfew	acetone	5	3	4	3	3	4
Chrysanthemum x-morifolium	Chrysanthemum of florists	acetone	10	4	1	0	4	0
Franseria acanthicarpa	False ragweed	acetone	5	2	3	0	4	0
Tanacetum vulgare	Tansy	acetone	10	3	4	4	4	2
Tribe: Heliantheae								
Ambrosia artemesifolia	Short ragweed	acetone	5	3	2	0	4	0
Gaillardia	Gaillardia	acetone	10	2	1	0	2	1
Helenium autumnale	Sneezeweed	acetone	10	4	0	0	4	1
Iva xanthifola	Burweed marsh elder	acetone	10	3	3	1	2	0
Rudbeckia hirta	Black-eyed susan	acetone	10	0	0	0	0	0
Xanthium commune	Cocklebur	acetone	10	3	2	0	3	0
Tribe: Inuleae								
Inula helenium	Alantolactone	ethyl alcohol 80% aqueous	0.2	3	2	1	4	4
Family: Frullanaceae								
Frullania nisquallensis	Liverwort	as is		4	1	4	2	3

Alantolactone (VII) produced positive patch test reactions in all five patients (Table I).

Patch tests were also carried out with extracts of species of Compositae. The results are shown in Table II. Some patch test reactions in Case 1 are shown in Fig. 5. Dr. A. K. Roy, Regina, Saskatchewan carried out patch tests with *Frullania nisquallensis* applied 'as is' in five patients who presented the clinical features of ragweek oil contact dermatitis and who showed positive reactions to ragweed 'oleoresin'. Positive reactions were observed in only two of the five cases.

One patient was patch tested with 12 *Frullania* species (as is) obtained by Dr. Redfearn from various geographic areas. Positive reactions were observed to 11 of the 12 species tested (Table III).

Using the method of Daniels (6) *Frullania* and alantolactone did not exhibit psoralen-type phototoxicity.

Investigation of Frullania nisquallensis *for allergenic activity. Frullania nisquallensis,* the most abundant of the seven species of *Frullania* found in British Columbia, was used for chemical analysis. Progress in isolation and purification of the active fractions was checked by patch testing of three patients whose cases (1, 4 and 5) are reported above.

As a first approach, we assayed solubility, polarity and volatility of the chemicals involved. All three patients were found reactive to organic solvent extracts of the plant material and especially to ethereal extracts, when the ether extract was examined by TLC with silica-gel as adsorbent, the allergenic activity was present in a narrow chromatographic band of low polarity. Steam distillates of this chro-

TABLE II

Patch test reactions to chemicals derived from Frullania *and Compositae*

Class	Number	Name	Vehicle 80% ethyl alcohol Concentration (mg/ml)	Patch test results Case				
				1	2	3	4	5
Eudesmanolides (santonin-type)	I	Not yet named	250	2			3	4
	II	Arbusculin-B	200	2			3	3
	III	Dihydroarbusculin-B	400	0			0	0
	IV	Not yet named	400	4			3	4
	V	Arbusculin-A	200	2			3	3
	VI	α-Santonin	1000	0			0	0
Eudesmanolides (Alantolactone-type	VII	Alantolactone	80	3	2	1	4	4
	VIII	Isoalantolactone	200	0		0	0	1*
	IX	Tetrahydroalantolactone	400	0			0	0
	X	Dihydroalantolactone	400	0			0	0
Guaianolides	XI	Cumambrin B	400	3			2	0
	XII	Cumambrin A	400	3			1	0
	XIII	Formylcumambrin B	400	4			2	0
	XIV	Tetrahydrocumambrin B	400	0			0	0
	XV	Dihydrocumambrin B	400	0			0	0
Pseudoguaianolides	XVI	Coronopilin	400	2			0	1
	XVII	Ambrosin	400	3			0	0

* Delayed.

matographic fraction yielded an oil to which all patients showed strong positive patch test reactions. These data suggested that the allergens might be terpenoids. Gas-liquid chromatography of the steam-distilled oil revealed the presence of several peaks necessitating further purification. The allergenic activity was finally attributed to two compounds. One is present in small amounts and has not yet been identified. The second has been identified as Compound I (Table I), a sesquiterpene lactone which is the same compound as that recently isolated from *Frullania tamarisci* by Ourisson *et al.* (7) in Strasbourg. By our method of isolation and purification 500 g of dried *Frullania* plant yielded about 20 mg of Compound I.

Allergenic properties of various sesquiterpene lactones from Compositae. Sesquiterpene lactones are commonly found in Compositae (8, 9) but only occur to a small extent outside this family of flowering plants We speculated that the allergenic properties of compound I were perhaps shared by some other sesquiterpene lactones and that this would provide a reasonable explanation for the sensitivity to *Frullania* and chrysanthemum observed in

TABLE III

Patch test reactions to various species of Frullania *plant applied 'as is' in Case 5. Patches were removed at 20 hours and reactions scored at 48 hours*

Species	Source	Patch test results
Frullania asagrayana Mont.	Arkansas	4
F. riparia Hamp.	Missouri	4
F. squarrosa (R.Bl. and N.) Dum.	Arkansas	0
F. dilatata (L.) Dum.	Finland	3
F. nisquallensis Sull.	California	4
F. tamarisci (L.) Dum.	Denmark	4
F. kunzei Lehm and Lindenb.	Missouri	3
F. eboracensis Gottsche	Michigan	2
F. franciscana Howe	Portland	4
F. inflata Gottsche	Missouri	4
F. bolanderi Aust.	Michigan	2
F. usamiensis Steph.	Japan	2

Cases 1, 2 and 4. Thus, three patients were tested with various sesquiterpene lactones which had been isolated from members of the Compositae by Professor T. A. Geissman and

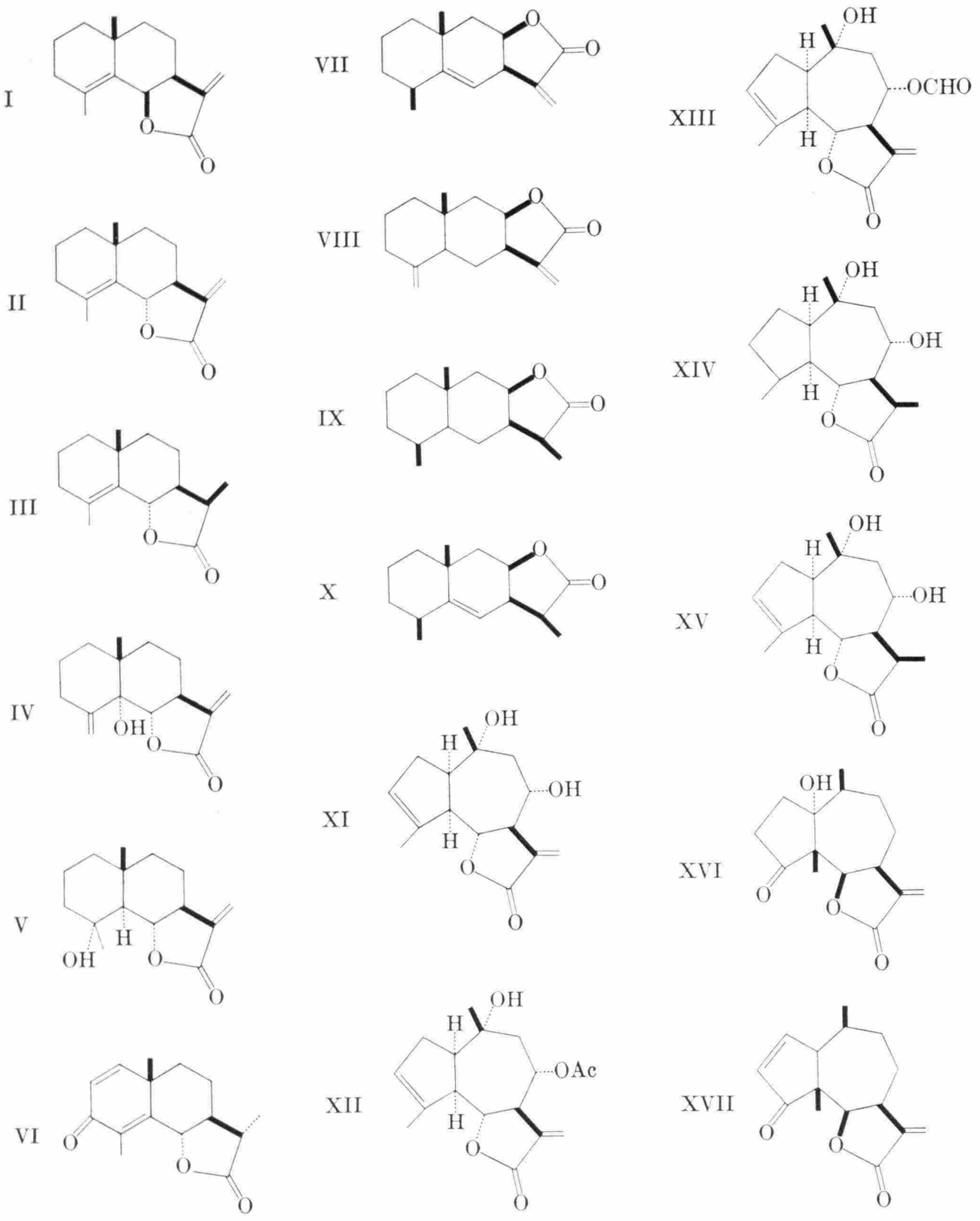

FIG. 3. Molecular structures of compounds named in Table II

which were kindly donated by him. The results are summarized in Table II and Fig. 3.

DISCUSSION

The results presented in Table II indicate that the allergenic properties of Compound I are shared by other sesquiterpene lactones because, among the 17 lactones tested, 10 gave positive reactions. The most allergenic lactone found so far is *not* Compound I which is present in *Frullania nisquallensis* and *Frullania tamarisci,* but alantolactone (VII), the oldest known sesquiterpene lactone. Its allergenic properties do not appear to have been reported previously. 0.6 μg of this compound applied to the skin of some patients by patch test was sufficient to produce a positive response within 12–24 hours.

The eudesmanolide class of lactones, both of the santonin type and of the alantolactone type, is of particular interest. These lactones produced positive patch test responses in all

three patients. On the contrary, the lactones of the guianolide and pseudoguianolide classes which were tested produced positive patch test responses in only one or two of the three patients. They were also less active at higher concentrations.

It is thought that haptens are probably bound to one or more proteins and, consequently, there may be some consistent structural requirement for the allergenic activity of these lactones. The active compounds noted above are represented in different classes and it may be observed that a specific skeleton is not a basic requirement for allergenic activity. Within each class, we selected lactones differing in only some simple functional group, such as an additional hydroxyl group, acetylation or formylation of a hydroxyl group, addition of an exocyclic or endocyclic carbon—carbon double bond, migration of a double bond, hydrogenation of one or two double bonds. For instance, compare (I) with (II), (III), (IV) and (V); (VII) with (VIII), (IX) and (X); (XI) with (XII), (XIII), (XIV) and (XV); (XVI) with (XVII).

We found only one common structural requirement, *viz.*, the presence of a conjugated lactone group. When the exocyclic carbon—carbon double bond conjugated with the carbonyl is reduced, there is a loss of allergenic activity; compare (II) with (III), (VII) with (IX) and (X), (XI) with (XIV) and (XV). However, the molecules possessing such a conjugated lactone are not all active: see (VIII). Nor does the stereochemistry at the level of the attachment of the lactone ring seem involved; compare (I), (VII), (XVI), (XVII) having an β,β attachment with (II), (IV), (V), (XI), (XII), (XIII) having an α,β attachment.

Evidently, some sesquiterpene lactones are probably the common denominators of allergic contact dermatitis from *Frullania* and Compositae species. More experiments are necessary to determine the exact structural requirements of such molecules. It appears that a conjugated carbon—carbon double bond is involved. This suggests that terpenoids other than lactones may play a similar role. We have already found evidence to support this hypothesis. Analysis of a *Chrysanthemum* species gave a volatile fraction which behaved on TLC like a hydrocarbon and to which patients reacted strongly. The identification of the allergens involved requires further study.

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