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SUBJECT: Applied chemotaxonomy of plants I. A biological model.

While phytochemists are amongst the most cooperative people now known, it is not always possible to persuade them to expend their research funds and staff's time in finding out the allergenic constituents of plants. A significant reason for this situation is that the work to be done is too easy; a properly equipped phytochemical laboratory can rapidly produce the required information but with tedious delays as the delay is at the clinical level and often results from lack of availability of sensitized subjects at suitable times. Furthermore younger phytochemists do not find career advancement in isolating a compound; if the compound turns out to be one new to biochemistry, a brief note in Chemical Abstracts may result but so what? If the compound turns out to be already known, the worker has wasted six months of his valuable time which he would have preferred to spend on more erudite subjects such as the study of biosynthetic pathways and so on. A partial solution to this problem is biological screening of plants for allergenic properties and the reason for the above rather pretentious title is to indicate that dermatologists have something to offer the chemotaxonomist who is interested in the distribution of compounds in plants.

The biological model consists of a small group of sensitized individuals who are willing to be patch tested to large numbers of plants in order to provide chemotaxonomic data which is not necessarily definitive but which may suggest further lines of detailed investigation. Since reference will be made to this report in subsequent reports, such sensitized individuals are delineated.

Three white adult males patch test negative to North American Contact Dermatitis Group Screening sets of 1972, 1973 and local additions to the set introduced from time to time except Case 3, positive to pyrethrum. All three negative to turpentine, turpentine peroxides (Trolle-Lassen) and to delta-3-carene. Cases 1 and 2 not atopic, Case 3 possibly atopic. The sensitivity level of the individuals to alantolactone (purified to remove isoalantolactone) was determined by patch testing to serial dilutions 50  $\gamma$ , 25  $\gamma$ , 10  $\gamma$ , 5  $\gamma$ , 2  $\gamma$ , 1  $\gamma$ , 0.5  $\gamma$ , 0.1  $\gamma$  and in Case 3 to 0.01  $\gamma$ , each applied in 20  $\mu$  ethanol.

One or more of these individuals, but usually Case 1 (2  $\gamma$ ) and Case 2 (0.5  $\gamma$ ) will be patch tested to plants and to plant products in order to serve as a biological screen for the presence of sesquiterpene lactones in plants. A positive patch test reaction indicates no more than that a plant or product tested may contain among its constituents or derivatives, a lactone in adequate amount to produce patch test dermatitis; a negative reaction,

of course, does not signify absence of such a compound. Controls, usually five in number, will be tested to all plants and products examined in order to detect irritancy. It is possible that repeated patch testing may lead to multiple specific sensitivity to other compounds in plants.

Case 1. A white adult male, aged 48 years, physician, had no clinical dermatitis but was sensitized to Frullania nisquallensis by serving as a previous negative control for contact sensitivity to this plant. Patch tests to the leaf, as is, of F. asagrayana, F. riparia, F. dilatata, F. nisquallensis, F. tamarisci, F. kunzei, F. eboracensis, F. franciscana, F. inflata, F. bolanderi, F. usamiensis, positive (++) : F. squarrosa, negative.

Marchantia polymorpha, Porella cordaema, Lunularia sp., negative. Acetone extracts (Hollister-Stier) of Chrysanthemum parthenium, Tanacetum vulgare, Helenium autumnale, Iva spp., mixed positive: Chrysanthemum x morifolium, C. maximum, C. cinerariifolium, Gaillardia pulchella, Franseria acanthicarpa, Ambrosia eliator, Parthenium hysterophorus, Artemisia absinthium, Achillea millefolium, Anthemis cotula, Rudbeckia hirta, Xanthium strumarium negative. Negative to other plant extracts of the Hollister-Stier Wild and Domesticated vegetation Patch test sets. Pyrethrum, turpentine, turpentine peroxides (Trolle Lassen), negative. Tincture of arnica, negative. Negative to isoalantolactone: positive to alantolactone (purified) in serial dilutions to 2.0  $\gamma$  in 20  $\mu$ l.

Patch test results to sesquiterpene lactones: ludovicin A, B and C ++, artemorin acetate ++, eupatolide acetate ++, matricarin -, eupatoriopicrin -, parthenolide ++, frullanolide ++, xanthinin -, chamissonin -, cumambrin A -, formylcumambrin B -, coronopilin +, cumambrin B -, ambrosin -, cumambrin -, dihydrocumambrin B -, mikanolide -.

Case 2. A white male, aged 64 years, retired carpenter-forest worker, had dermatitis of the hands, forearms, face and neck. He had been obliged to retire from forest work because of recurrent dermatitis from Frullania and, as a retirement hobby, took up growing chrysanthemums to which, he found, he was contact-sensitive.

Patch test results were as follows: - Acetone extracts (Hollister-Stier) of Chrysanthemum x morifolium, Chrysanthemum parthenium, Tanacetum vulgare, Gaillardia pulchella, Franseria acanthicarpa, Ambrosia eliator, Helenium autumnale, Parthenium hysterophorus, Ambrosia absinthium, Achillea millefolium, Iva spp. mixed, Anthemis cotula, Xanthium strumarium positive: Chrysanthemum cinerariifolium, Chrysanthemum maximum, Rudbeckia hirta, Cosmos, Coreopsis negative. Negative to other plant extracts of the Hollister-Stier Wild and Domesticated vegetation Patch test sets. Pyrethrum, turpentine, turpentine peroxides (Trolle Lassen) negative. Leaf of Frullania nisquallensis, as is positive. Negative to isoalantolactone: positive to alantolactone (purified) in serial dilutions to 0.5  $\gamma$  in 20  $\mu$ l.

Negative to ethanol extract of Cirsium, Erythronium montanum, Tulipa, and to  $\alpha$ -methylene  $\gamma$ -butyrolactone. He was patch tested to a leaf of herbarium specimens of Compositae species (the first 47 plants in alphabetical order) and showed positive patch test reactions to Apargidium boreale, Arnica cordifolia, A. latifolia, Artemisia lindleyana, A. ludoviciana, A. michauxiana, A. suksderfii, A. trifida negative to the other 38 species.

Patch test results to sesquiterpene lactones: Frullanolide +++, coronopilin -, ambrosin -, cumambrin ++, dihydrocumambrin B -, tetrahydrocumambrin B -, xanthinin-, chamissonin -, cumambrin A +, formylcumambrin B ++, santonin -, ludovicin A, B and C ++, artemorin acetate +, eupatolide acetate ++, eupatoriopicrin +, matricarin -.

Case 3. A white male, aged 75 years, retired electrician, active gardener, had dermatitis of the hands, forearms, face and neck. The prime cause of his dermatitis was growing chrysanthemums as a hobby. Patch test results were as follows: - Acetone extracts (Hollister-Stier) of Chrysanthemum x morifolium, Chrysanthemum parthenium, Chrysanthemum cinerariifolium, Tanacetum vulgare, Gaillardia pulchella, Franseria acanthicarpa, Ambrosia eliator, Helenium autumnale, Achillea millefolium, Iva spp. mixed, Anthemis cotula, Xanthium strumarium positive: Parthenium hysterophorus, Artemisia absinthium, Chrysanthemum maximum, Rudbeckia hirta, Helianthus annuus, Cosmos, Coreopsis negative. Negative to other plant extracts of the Hollister-Stier Wild and Domesticated vegetation Patch test sets. Pyrethrum positive; turpentine and turpentine peroxides (Trolle-Lassen) negative, Tincture of arnica negative. Leaf of Frullania nisquallensis, as is, positive. Negative to isoalantolactone: positive to alantolactone (purified) in serial dilutions to 0.016  $\gamma$  in 20  $\mu$ l.

Patch tests to sesquiterpene lactones: Spathulin -, ivasperin ++, flexuosin B -, pulchellin C -, isotenulin -, baldwinin -, tenulin  $\pm$ , gaillardilin -, pyrethrosin ++, parthemollin -, pseudoivalin +++, helenalin +++, pinnatifidin +++, ivalin +++, parthenolide +++, tetraeurin D -, ambrosiol +++, conchosin A -, psilostachyin B ++, isabelin -, tamaulipin B +++, damsine +++, psilostachyin +, psilostachyin C -, tamaulipin A +++, elephantopin -, vernolepin  $\pm$ , vernomenin  $\pm$ , reynosin +++, pulchellin C -, cnicin -, desacetylconfertiflorin -, tetraeurin E +++, frullanolide ++, ambrosin +++, cumambrin ++, dihydrocumambrin -, tetrahydrocumambrin B -, xanthinin  $\pm$ , chamissonin -, cumambrin A +++, formylcumambrin B +++, coronopilin ++, santonin -:and delta-3-carene -.

Case 3. Results of patch tests to cultivars of Chrysanthemum x morifolium,  
in Case 3.

TABLE

Section	Cultivar Name	Colour	Leaf, applied as is	Patch test results *
24 (a)	Bonanza	Red		+++
24 (b)	Broadway	Purple		++
29 (c)	Charming	Pale Pink		-
25 (b)	Cricket	White		-
25 (b)	Cricket	(White)- Yellow Sport		-
24 (b)	Early Red Cloak	Red		++
28 (a)	Fairie	Pink		+++
25 (a)	Gladys Sharpe	Yellow		++
25 (b)	Golden Charm	Light bronze		++
** 24 (a)	?Golden Rule	Yellow		++
25 (a)	Gordon Johnson	Light bronze		+
25 (b)	Graham Knight	Bronze		++
24 (a)	Grandstand	Bronze		++
24 (a)	Headliner	Pink		+++
25 (a)	Jack Curtis	Yellow		++
25 (a)	Jack Truswell	White		-
24 (b)	Marshmoor Glory	(Light bronze)- White sport		+
23 (b)	Nancy Mathews	White		+
25 (a)	Goldington Queen	(White)-Pink sport		++
10	Rayonnante	Pink		++
25 (a)	Smilin Thro'	Pink		++
24 (a)	Standard	Bronze		-

TABLE (con'd)

Section	Cultivar Name	Colour	Leaf, applied as is	Patch test results *
25(b)	Tombola	Light bronze		++
Tombola is classified 25(b) - Medium flowered, but this plant was large flowered as are plants of 25(a).				
24(a)	Tom Briggs	White		++
24(a)	Tracy Waller	Pink		++
25(a)	Westfield Bronze	Bronze		-

\* Patch removed at 48 hours, reaction read at 96 hours. Scored by method of Wilkinson et al (1970).

\*\* Identification of cultivar uncertain

Section 10: Spidery, etc.

Section 23: Incurved Decoratives (a) Large-flowered, (b) Medium-flowered.

Section 24: Reflexed Decoratives (a) Large-flowered, (b) Medium-flowered.

Section 25: Intermediate Decoratives (a) Large-flowered, (b) Medium-flowered.

Section 29: Sprays (a) Anemones, (b) Pompons, (c) Reflexing, (d) Singles.

Gosling S.G. (1969). The Chrysanthemum Manual. 3rd. ed. Appendix VII Classification of Chrysanthemums by the Floral Committee of The National Chrysanthemum Society p. 325. The National Chrysanthemum Society, 65 St. Margaret's Ave., Whetstone, London N20.

Stevenson J B and Gosling S G (1968). Chrysanthemum Stopping and Timing. 2nd ed. The National Chrysanthemum Society. 65 St. Margaret's Ave., Whetstone. London N20.

Since the patient (Case 3) may not be readily available, a fourth individual has been added to the panel: -

Case 4. A white male forest-worker, aged 55 years, had attacks of dermatitis affecting the hands, forearms and face during work amongst vegetation 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 whilst at home. This history is typical for some 55 Frullania contact sensitive forest-workers in British Columbia. Patch tests were positive to Frullania spp. and to allantolactone (++, 48 and 72 hours). The sensitivity level of this patient to allantolactone was determined, as in the other patients and the results are shown in the Table. He will be designated Case 4 (0.08  $\checkmark$ ).

Weight of alantolactone applied in 20	<u>Time - Patch Test Result</u>						
	24 hrs.	48 hrs.	72 hrs.	96 hrs.	5 days	6 days	7 days
50	++	++	++	++	+++	+++	+
25	++	++	++	+++	+++	+++	+
10	++	++	++	++	+++	+++	++
5	++	++	++	++	+++	+++	++
2	++	++	++	++	+++	+++	+
1	+	+	+	++	++	++	+
0.4	±	+	+	+	+	+	+
0.2	±	+	+	+	±	±	±
0.08	+	±	±	+	±	±	±
0.04	-	-	-	-	-	-	-
0.016	-	-	-	-	-	-	-

Case 4. Tests for sensitivity level to alantolactone (purified).