Phytochemistry, 1973, Vol. 12, pp. 811 to 815. Pergamon Press. Printed in England.

TULIP ALLERGENS IN *ALSTROEMERIA* AND SOME OTHER LILIIFLORAE

A. SLOB

Netherlands Institute for Preventive Medicine TNO, Wassenaarseweg 56, Leyden, The Netherlands

(*Received* 15 *October* 1972. *Accepted* 8 *November* 1972)

Key Word Index—*Alstroemeria*; Liliaceae; relationship among Liliiflorae; allergens; tuliposides; chemotaxonomy.

Abstract—A study has been made of the occurrence of tulip allergens (tuliposides) among several plant genera belonging to the Liliiflorae. All species of the genera *Alstroemeria*, *Erythronium* and *Tulipa* can be considered potentially allergenic (tuliposide-A). Tuliposide-B is more generally distributed and occurs in *Lilium*, *Notholirion* and *Calochortus* as well. Small amounts of both tuliposides are found in *Fritillaria*. A brief discussion is given of the systematic implications of the results.

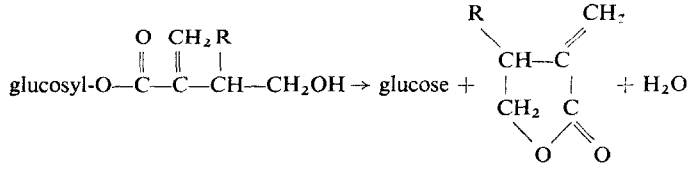
INTRODUCTION

TULIPS contain antibiotic[1,2] and allergenic[3] substances in the bulbs, leaves and flowers. Five years ago, two independent research teams[4,5] succeeded in isolating α-methylene-γ-butyrolactone from tulip bulbs. This compound had already been obtained from *Erythronium americanum*.[6] Bergman *et al.*[4] recognized the strong fungitoxic activity of this unsaturated lactone, whereas Verspyck Mijnssen[3] proved it to be the causative agent of an allergic skin disease, known in Holland as 'tulpenvinger' (tulip finger). Tschesche *et al.*[7] isolated two bacteriotoxic and fungitoxic glucose esters from tulip leaves and flowers, which they called tuliposide-A and tuliposide-B. Both tuliposides hydrolyse easily into glucose and an unsaturated lactone under weak alkaline conditions. In the case of tuliposide-A this lactone proved to be α-methylene-γ-butyrolactone, and in the case of tuliposide-B its β-hydroxy-derivative (Scheme 1). Both lactones show a strong antibiotic activity, but the allergenic properties seem to be restricted to α-methylene-γ-butyrolactone.[8]

From the above studies, it became clear that precursors of biologically active lactones are present in two genera of Lilioideae sensu Buxbaum[9] (or Liliaceae sensu Huber[10]).

[1] OSBORNE, E. M. (1943) *Br. J. Exp. Path*, **24**, 227.
[2] SCHÖNBECK, F. (1967) *Phytopath. Z.* **59**, 205.
[3] VERSPYCK MIJNSSEN, G. A. W. (1968) Thesis, Leiden.
[4] BERGMAN, B. H. H., BEIJERSBERGEN, J. C. M., OVEREEM, J. C. and KAARS SIJPESTEIN, A. (1967) *Rec. Trav. Chim.* **86**, 709.
[5] BRONGERSMA-OOSTERHOFF, U. W. (1967) *Rec. Trav. Chim.* **86**, 705.
[6] CAVALLITO, C. J. and HASKELL, T. H. (1946) *J. Am. Chem. Soc.* **68**, 2332.
[7] TSCHESCHE, R., KÄMMERER, F. J. and WULFF, G. (1968) *Tetrahedron Letters* **6**, 701; *idem.* (1969) *Chem. Ber.* **104**, 2057.
[8] SLOB, A. (1972) unpublished results.
[9] BUXBAUM, F. (1937) *Botan. Arch.* **38**, 213, 305, 338.
[10] HUBER, H. (1969) *Mitt. Botan. München* **8**, 219.

Further research into this field seemed worthwhile, when observations of an allergic skin disease similar to the 'tulip finger' allergy, but caused by *Alstroemeria* species, were communicated to Verspyck Mijnssen.



R = H, Tulíposide-A R = H, α-methylenebutyrolactone,
R = OH, Tuliposide-B R = OH, α-methylene-β-hydroxybutyrolactone

SCHEME 1.

In dermatology, a sound knowledge of allergenic plants and their active principles is of great importance. Identification of allergenic substances will faciliate the prophylaxis and therapy of diseases. In addition these tuliposides may become of value to plant systematics, once their distribution is better known.

TABLE 1. TULIPOSIDES IN LILIIFLORAE (ARRANGEMENT OF TAXA ACCORDING TO HUBER)

LEP No.	Plant	Tuliposide-A				Tulíposide-B	
		Flower		Leaves		Flower	Leaves
		PC	GLC	PC	GLC	GLC	GLC
	COLCHICOID LILIIFLORAE						
	LILIACEAE s. str.:						
	GAGEA						
	lutea L.	−	−	−	−	+	−
	ERYTHRONIUM						
21708	*dens-canis* L.			±	+		−
20461	*revolutum* Sm.	+	++	+	++	++	++
20462	*tuolomnense* Applegate	+	+	+	+	++	++
	TULIPA						
20475	*acuminata* Vahl.	+	++	++	++	+	+
20476	*batalinii* Reg.	−		−			
20477	*biflora* L.f.	+		+			
20478	*clusiana* Vent.	+	++	+	++	+	++
20479	*eichleri* Reg.	±	±	±		++	
20480	*greigii* Reg.	±		±			
20481	*hageri* Heldr.	++		+			
20482	*kolpakowskyana* Reg.	+		+			
20483	*linifolia* Reg.	++		+			
20484	*maximowiczii* Reg.	++		+			
20485	*orphanidea* Boiss.	+		++			
20486	*praecox* Ten.	++	++	++	+	+	+
20487	*praestans* Hoog	++		+			
20488	*saxatilis* Sieb.	++	++	++	+	±	++
20489	*stellata* Hook.	+		++			
20490	*tarda* Stapf.	+		++			

TABLE 1—*continued*

LEP No.	Plant	Tuliposide-A				Tuliposide-B	
		Flower		Leaves		Flower	Leaves
		PC	GLC	PC	GLC	GLC	GLC
	FRITILLARIA						
20463	*assyriaca* Bak.	±	−	±	+		+
20464	*imperialis* L, (cultivar 'Orange Brilliant')	+	±	−	−	−	−
20465	*meleagris* L. (cultivar 'Aphrodite')	±	±	+	−	−	−
20466	*meleagris* L. (cultivar 'Saturnus')		±		−	−	+
20467	*persica* L.	±	±	±	−	±	−
	NOTHOLIRION						
21709	*thomsonianum* Stapf.			+	−		++
	LILIUM						
20469	*amabile* Palibin	±	−	+	−	−	+
20471	*henryi* Bak.	±	−	−	−	++	+
20474	*regale* Wils.	−	−	−	−	++	±
20472	*tenuifolium* Fisch.	−	±	−	−	−	−
20470	*willmottiae* L.	−	−	−	−	−	−
	ALSTROEMERIACEAE: *ALSTROEMERIA*						
20459	*aurantiaca* Don	++	++	+	+	±	+
20460	*ligtu* L.	+	++	±	+	+	+
	CALOCHORTACEAE: *CALOCHORTUS*						
20492	*uniflorus* Hook. et Arn.	+	±	±	−	+	++
	ASPARAGOID LILIIFLORAE						
	TECOPHILAEACEAE: *TECOPHILAEA*						
20491	*cyanocrocus* Leichtl.	±	±	−	−	+	−
	ASPHODELACEAE: *KNIPHOFIA*						
	cf. *natalensis*			−	−		−
	AGAVACEAE: *HOSTA*						
	cf. *sieboldiana*			−	−		−
	ALLIACEAE: *ALLIUM*						
20494	*schoenoprasum* L.			−	−		−

Key. − not detectable; ± present in trace amounts; + present in moderate amounts; ++ present in large amounts.

There are some indications that among the Liliiflorae, morphological evolution was often accompanied by chemical evolution (thus colchicine-like alkaloids seem to be present in Colchicaceae sensu Huber only, whereas the presence of the alkaloids which are biogenetically related to belladine seems to be restricted to Amaryllidaceae s. str.[11]).

[11] HEGNAUER, R. (1963) *Chemotaxonomie der Pflanzen*, Vol. 2, Birkhäuser, Basel.

RESULTS AND DISCUSSION

All plants so far studied by us for the presence of tuliposide-A and -B are listed on Table 1. The work of Buxbaum[9,12] and Huber[10] contributed substantially to our understanding of Liliales or Liliiflorae. Both authors have revised the classification of this group of plants. According to them, Alstroemeriaceae, for instance, are closely related to the Liliaceae–Lilioideae complex. Since Alstroemariaceae are hypogynous plants, they are also very often associated with Amaryllidaceae.[13,14] Huber[10] suggested a more natural classification comprising four evolutionary lines for the Liliiflorae: dioscoreoid, colchicoid, asparagoid and haemodoroid Liliiflorae (Fig. 1). The colchicoid line, which he linked with the dioscoreoid line through Medeola and Scoliopus, consists of, e.g. Colchicaceae (Wurmbaeoideae + Uvularieae sensu Buxbaum), Iridaceae, Alstroemeriaceae, Tricyrtidaceae, Liliaceae s.str (Liliaceae–Lilioideae sensu Buxbaum), Calochortaceae and Melanthiaceae (Melanthieae, Helonideae and Narthecieae) (Fig. 2). According to his classification, the Amaryllidaceae belong to the asparagoid Liliiflorae, however.

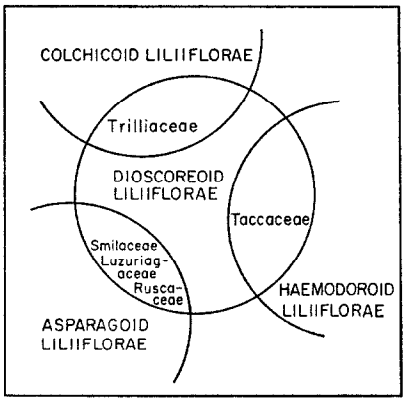


FIG. 1. THE FOUR EVOLUTIONARY LINES OF LILIIFLORAE ACCORDING TO HUBER.[10]

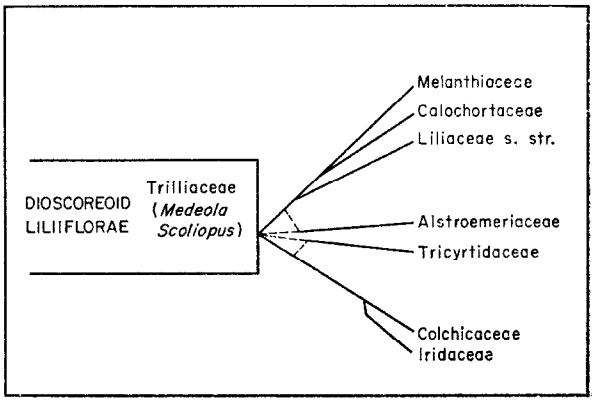


FIG. 2. EVOLUTIONARY LINES IN COLCHICOID LILIIFLORAE ACCORDING TO HUBER.[10]

From the results reported in Table 1, some preliminary conclusions can be drawn. All species of the genera *Alstroemeria*, *Erythronium* and *Tulipa* can be regarded as potentially allergenic plants. Nearly all species examined contained considerable amounts of tuliposide-A, usually accompanied by varying amounts of tuliposide-B. Tuliposide-A appeared to be a common metabolite of certain *Fritillaria* species as well, although it occurred in much smaller amounts. In general, tuliposide-B appeared to occur more often in easily detectable amounts than tuliposide-A. In addition to its being present in the genera mentioned, we found it in rather large amounts in species of *Lilium*, in *Notholirion thomsonianum* and in *Calochortus uniflorus*. By means of GLC techniques we found it to be present in the flowers of *Gagea lutea* and *Tecophilaea cyanocrocus* as well.

A truly systematic evaluation of this biochemical characteristic will only be possible after a comparative analysis of more taxa of Liliiflorae has been made. We nevertheless

[12] BUXBAUM, F. (1954) *Österr. Botan. Z.* **101**, 337.
[13] PAX, F. and HOFFMANN, K. (1930) *Amaryllidaceae, in Engler-Prantl, Die natürliche Pflanzenfamilien*, 2nd Edn, Vol. 15a, Engelmann, Leipzig.
[14] HUTCHINSON, J. (1959) *The Families of Flowering Plants*, 2nd Edn, Vol. II, Clarendon Press, Oxford.

obtained some evidence pointing towards close biochemical similarities between Lilioidaea (Liliaceae *sensu* Huber) and Alstroemeriaceae. This system (tuliposides → antibiotic lactones), which is probably involved in the disease resistance of the plants concerned, is mainly realized in the two taxa mentioned. Of course, we also detected tuliposide-B in other taxa, which points towards a wider distribution of tuliposides. In order to make more detailed conclusions possible, a wider survey is called for.

EXPERIMENTAL

Plant material. Most plants were obtained from commercial sources (Tubergen, Haarlem) and cultivated in the garden of the Laboratory of Experimental Plant taxonomy of the State University Leyden (LEP). Voucher specimens were deposited in the Herbarium of the LEP. Some species (no voucher specimens) were obtained from the botanical garden of the Leyden University. *Gagea lutea* was collected in Switzerland and cultivated in Leyden.

Extraction. 1 g of fresh plant parts was cut into small pieces and then boiled for 0·5 hr in 50 ml H_2O. By this procedure, a weak acid milieu was maintained (pH $\leqslant$ 6), in which the tuliposides are reasonably stable. The filtrate was collected and taken to dryness on a steam bath after a mixture of sand and diatomite (Hyflo) had been added. The dry residue was then mechanically shaken for 4 hr with 5 ml EtOH. After centrifugation, the supernatant was again taken to dryness. The residue was extracted with 0·2 ml EtOH ($\times$3) and then stored in a refrigerator. The extracts thus obtained are reasonably stable, though some decomposition occurs with time. This decomposition may account for some of the discrepancies between the results obtained by PC and GLC. The more sensitive GLC procedure was carried out at a later stage, when decomposition might already have occurred. PC techniques were designed for the detection of the allergenic precursor (tuliposide-A). Ascending and descending techniques (on Schleicher and Schüll 2043 bMgI paper) were used. As a routine solvent, *n*-BuOH–Me_2CO–H_2O (5:1:2) was chosen (tuliposide-A R_f 0·44). Tuliposide-A was localized on chromatograms by ammoniacal $AgNO_3$[15] (Tollens reagent). During GLC we also examined for the presence of tuliposide-B. The extracts were silylated with bis-(trimethylsilyl)-acetamide (BSA). 0·1 ml of extract was taken to dryness and dissolved in 0·3 ml dimethylformamide and 0·2 ml of BSA, after which the solution was kept air-tight at 45° for 0·5 hr. Finally, 3 μl was injected on the gaschromatograph at 230° (column 1·8 m, 5% SE-52 on Chromosorp-W 100/120 mesh, N_2-flow 30 ml/min, FID detection).

Acknowledgements—Our thanks are due to Professor Dr. R. Hegnauer for his interest and encouragement and for putting his laboratories at our disposal. Mr. J. Kyne prepared the extracts and performed the PC. Valuable technical assistance was rendered by Miss M. Hennis (GLC).

[15] MERCK, E. (1959) *Anfärbereagenzien für die Papierchromatographie*, Reagent 15B, Merck, Darmstadt.