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ON THE OCCURRENCE OF TULIPOSIDES IN THE LILIIFLORAE

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Key Word Index—*Alstroemeriaceae*; Liliaceae; Relationships among Liliiflorae; allergenic plant constituents; post-inhibitins; tuliposides; chemotaxonomy.

Abstract—Approximately 200 samples of liliiflorous plants were investigated for the presence of tuliposides. Appreciable amounts of tuliposide A were detected in all species of *Erythronium*, *Tulipa*, *Gagea*, *Bomarea* and *Alstroemeria*. Large amounts of tuliposide B seem to be restricted to *Erythronium* and *Tulipa*. The occurrence of identical post-inhibitins in *Tulipa* and allied taxa and in *Alstroemeria* and allied taxa is interpreted as indicating a close relationship between Lilioideae and Alstroemeriaceae. At the same time the allergenic potentialities of all taxa of *Alstroemeria* are stressed.

INTRODUCTION

Tuliposide-A, the ester of glucose with α-methylene-γ-hydroxybutyric acid, occurs in large amounts in tulips. It is accompanied by tuliposide-B, the β-hydroxyderivative of the former [1]. The hydroxy-acids released by enzymic or spontaneous hydrolysis of tuliposides lactonize easily to the corresponding α-methylenebutyrolactones under non-alkaline conditions [1, 2]. In recent times much attention has been paid to these tulip constituents by phytopathologists [3] and dermatologists [4] because they have strong fungitoxic and allergenic properties. For dermatologists, tuliposide-A and its reaction products seem much more important than tuliposide-B, because the allergic skin disease caused by tulips seems to be caused by α-methylene-γ-butyrolactone derived from tuliposide-A; in this respect the β-hydroxyderivative seems to be inert [5]. Tuliposide-A is present in considerable amounts in bulbs, stems, leaves and flowers of cultivated tulips.

In a recent publication, we reported results of a preliminary screening of liliaceous and related plants for tuliposides [5]. We stressed the need for more precise information about the distribution of tuliposide-A, the precursor of the allergenic lactone, because many liliiflorous taxa are cultivated on a commercial scale as ornamental plants. We also draw attention to the possibility that accumulation of tuliposides may represent a character of interest to plant systematics. The preliminary nature of the results and conclusions was stressed because the number of taxa investigated was small.

Meanwhile we have extended our coverage of taxa and considerably improved our analytical procedures. The present paper summarizes the results of a more elaborate study of the distribu-

✠ Arie Slob was the victim of a tragic accident in May 1974. This posthumous publication pays honor to an enthusiastic scientist and honest man, who is no longer with us. The final version of this article was drafted by Prof. R. Hegnauer (Laboratory for Experimental Plant Systematics, 5e Binnenvestgracht 8, Leiden, The Netherlands), to whom request for reprints should be addressed, Mrs. B. M. E. von Blomberg-v.d.Flier (Netherlands Institute for Preventive Medicine, TNO, Leiden) and Prof. Dr. H. L. Booy (Laboratory for Medical Chemistry, Leiden).

† Technical assistance.

‡ Cultivation and documentation of plants.

tion of tuliposides and includes some revisions of our previous findings.

RESULTS

Almost all plants available to us were investigated during the flowering stage. Each sample was divided in flowers, stems, leaves and underground parts (i.e. roots, rhizomes, bulbs). In each instance presence or absence of tuliposides was investigated by three analytical procedures, PC, TLC and GLC. In plant parts apparently containing tuliposides, the amounts present were deter-

Table 1. Taxa investigated for the presence of tuliposides, arranged according to the classification of Huber [6]

Main groups of Liliiflorae	Family	Taxa investigated*
Dioscoreoid Liliiflorae: Dioscoreales† Roxburghiales	Dioscoreaceae	*Dioscorea* cf. *hispida* 22638, *D.* cf. *quaternata* 22706, *D.* cf. *villosa* 22640, *D.* sp. 22639; *Tamus communis* 23068
	Trilliaceae†	*Medeola virginiana* 23314; *Paris quadrifolia* 23016; *Scoliopus bigelovii* 22665; *Trillium erectum* 23414, *T. grandiflorum* 23413, *T. luteum* 23414, *T. sessile*, *T. stylosum* 23411, *T. undulatum*
	Ruscaceae†	*Ruscus aculeatus* 22701
	Convallariaceae†	*Convallaria majalis* 22633; *Majanthemum bifolium* 22693, 23061; *Polygonatum* sp. 20728, *P. multiflorum* 23063 *P. odoratum* 20368, *P. verticillatum* 23019; *Smilacina racemosa*; *Streptopus amplexifolius* 23047
Asparagoid	Asparagaceae†	*Asparagus* sp. 8370, *A.* sp. 8373
	Tecophilaeaceae†	*Tecophilaea cyanocrocus* cv. violacea 23419, *T. cyanocrocus* cv. leichtlinii 20491
	Anthericaceae†	*Anthericum liliago* 20702, 23024, *A. ramosum* 23119; *Paradisia liliastrum*
Liliiflorae: Asparagales	Asphodelaceae†	*Asphodeline liburnica* 21434, *A. lutea* 22627; *Asphodelus microcarpus* 20731; *Bulbine annua* 21796; *Haworthia batesiana* 22651, *H. planifolia* cv. variegata 22652; *Kniphofia* × *pfitzeri*
	Agavaceae	*Hosta* cf. *elata* 22705, *H.* cf. *fortunei* 22697, *H.* cf. *glauca* 20713
	Hemerocallidaceae	*Hemerocallis dumortieri*, *H. lilio-asphodelus* 22702, *H.* cv. 22696, 22698
	Alliaceae	*Allium coeruleum* (= *A. azureum*) 22704, *A. cepa* 23421 *A. karataviense* 22614, *A. sativum* 23576, *A. ursinum* 22615; *Brodiaea* cf. *hyacynthina* 22628, *B.* cf. *peduncularis* 22629, 22630, *B. terrestris* 23574, *B.* sp. 23573; *Ipheion uniflorum* 22653; *Nothoscordum inodorum* (= *N. fragrans*) 22662
	Hyacinthaceae†	*Lachenalia aloides* 23392; *Muscari armeniacum* 23420, *M.* sp. 22658; *Ornithogalum balansae* 22663, *O.* cf. *longibracteatum* 22721, *O.* sp. 22664; *Veltheimia viridiflora* (= *V. capensis*) 23429
	Amaryllidaceae	*Galanthus elwesii* 22645, *G. ikariae* 22646, *G. nivalis* 22647; *Leucojum aestivum* 22657, *L. autumnale* 22313, *L. vernum* 23386; *Narcissus bulbocodium* 22659, *N. Jonquilla* 22660, *N. nanus* (= *N. lobularis*), *N. asturiensis* (= *N. minimus*) 22661; *Pancratium maritimum*; *Sternbergia colchiciflora* (= *S. clusiana*) 22666, *S. lutea* 22667
	Colchicaceae†	*Bulbocodium vernum* 23416, *Colchicum autumnale* 22631 *C. byzantinum* 22869, *C. speciosum* 22632; *Gloriosa superba* 22650, *G. virescens* cv. Rothschildiana 22648, cv. 22649; *Uvularia grandiflora* 22679, *U. perfoliata*
Colchicoid Liliiflorae:	Iridaceae	*Crocus ancyrensis* 22634, *C. medius* 22870, *C. speciosus* 23384, *C. susianus* 22635, *C. tomasianus* 22636; *Gladiolus byzantinus* 22703, *G. segetum* 23575; *Iris bucharica*, *I. florentina* 22654, *I. graminea* 22655, *I. pseudacorus* 22700, *I. reticulata* 23415, *I. sibirica* 22656, *I. versicolor* 23430; *Tigridia pavonia*.

Table 1. Continued.

Main groups of Liliiflorae	Family	Taxa investigated*
Liliales	Alstroemeriaceae†	*Alstroemeria aurantiaca* 22617, *A. gayana* 22618, *A. haemantha* 22619, *A. hookeri*, *A. inodora* 22620, *A. ligtu* 20460, *A. pelegrina* 22621, *A. philippii*, *A. psittacina* (= *A. pulchella*) 21378, 22622, *A. pulchra* (= *A. ligtu* var. *pulchra*) 22623, *A. revoluta* 22625, *A. versicolor*, *A. violacea* 22626, *A.* taxa 22624, 22695, 22616, *A.* "Aalsmeer 4420"; *Bomarea cardieri*, *B. edulis*, 23595, *B.* sp. 23596
	Tricyrtidaceae†	*Tricyrtis hirta* 22677, 22694, *T. latifolia* 22678, *T. stolonifera* (= *T. formosana*) 23315
	Liliaceae†	*Erythronium dens-canis* cv. Liliac Wonder 21708, *E. revolutum* 20461, *E. tolumnense* 20462, *E.* cv. kondo, *E.* cv. Pagoda 23428; *Gagea fistulosa* 19869, *G. lutea* 5956, 19617A, 19854, 23418, *G. minima* 19857, *G. saxatilis* 22644; *Fritillaria acmopetala* 23417, *F. armena* 23593, *F. assyriaca* 22641, *F. imperialis* 20464, *F. meleagris*, *F. nigra* (=*F. tenella*) 22642, *F. persica* 22643, *F. pontica*, *F. pyrenaica*, *F. schliemannii*; *Lilium amabile* 20469, *L. bulbiferum* 23120, *L. davidii* var. *willmottiae* 20470, *L. henryi* 20471, *L. martagon* 23073, *L. pumilum* 23422, *L. regale* 20474, *L.* sp. 20468; *Notholirion thomsonianum* 21709; *Tulipa acuminata* 23423, *T. batalinii* 22668, *T. biflora* 23427, *T. celsiana*, 22669, *T. clusiana* 23425, *T. didieri* 22671, *T. eichleri* 23424, *T. kolpakowskyana* 23426, *T. mauritiana* 22672, *T. stellata* var. *chrysantha* 22670, *T. turkestanica* 22673, *T. urumiensis* 22674 *T. whittallii* 22675
	Calochortaceae†	*Calochortus uniflorus* 20492
	Melanthiaceae†	*Narthecium ossifragum* 22706; *Tofieldia calyculata* 22676, 23018, *Veratrum album* 23012, *V. nigrum* 22680 *Zygadenus elegans* 22699

* Numbers after plant names are numbers of voucher specimens deposited together with color slides in the herbarium of "Laboratorium voor Experimentele Plantensystematiek, Leiden". If no LEPS-number are given all material available was used for chemical examination.

The plants investigated were received from different sources as follows:

A. Botanical Gardens of the Universities of Leiden, Amsterdam and Utrecht and of the Technical University of Delft and the Agricultural University of Wageningen. Messrs H. J. van Hattum (Leiden) and E. Mennega and J. Tolsma (Utrecht) were most helpful (identification, documentation).

B. Samples of *Gloriosa* came from Philips-Duphar, 's-Gravenland.

C. Most samples of Alstroemeriaceae were provided by "Proefstation voor de Bloemisterij" (Experimental Station for Floriculture), Aalsmeer, through the kind collaboration of Ir. C. Vonk Noordegraaf.

D. Commercial Firms; especially van Tubergen, Haarlem, whose director, Mr. H. M. Hoog kindly advised us in several respects.

E. Several species were collected in nature by ourselves (Holland, Switzerland) or received by the kind collaboration of botanists: *Scoliopus* (Prof. Dennis E. Anderson, Humboldt State College, Arcata, California); *Medeola* (Prof. Richard H. Eyde, Smithsonian Institution, Washington; Lieutenant James B. McMullin, amateur naturalist, Lancaster, Pennsylvania).

F. A number of North American plants were purchased from a commercial grower, offering wild species (Francis M. Sinclair, New Hampshire 03833, U.S.A.).

† For the convenience of the reader the corresponding taxa in the treatment of Liliiflorae by Melchior [7] are given here: Trilliaceae = Liliaceae-Parideae; Ruscacacae = Liliaceae-Asparageae p.p.; Convallariaceae = Liliaceae-Convallarieae and Polygonateae; Asparagaceae = Liliaceae-Asparageae p.p.; Tecophilaeaceae = Haemodoraceae-Conanthereae p.p.; Anthericaceae = Liliaceae-Asphodeleae p.p. and -Johnsonieae; Asphodelaceae = Liliaceae-Asphodeleae p.p. and -Aloeae; Hyacinthaceae = Liliaceae-Scilloideae and -Bowieae; Colchicaceae = Liliaceae-Wurbaeoideae and -Uvularieae; Alstroemeriaceae = Liliaceae-Alstroemerioideae; Tricyrtidaceae = Liliaceae-Melanthoideae-Tricyrteae; Liliaceae = Liliaceae-Lilioideae (excl. Calochorteae); Calochortaceae = Liliaceae-Lilioideae-Calochorteae; Melanthiaceae = Liliaceae-Melanthioideae (excl. Uvularieae and Tricyrteae).

Table 2. Tuliposide contents of plants whicl

Taxon*	Tuliposide A											
	GLC				PC				TLC			
	F†	L†	S†	B†	F	L	S	B	F	L	S	B
Alstroemeria aurantiaea	++	++	±	++	+++	+++	−	+++	+++	++	+	+++
A. gayana	+++	+	++	++	++++	++	++	+++	+++	+	++	++
A. haemantha	+++	+	++	++	+++	++	+++	+++	+++	++	+++	++
A. hookeri	+++	++++	+++	+	+++	++++	+++	−	++	+++	++	+
A. inodora	+++	++	++	+	+++	+++	++	++	+++	+++	++	+
A. ligtu 20460	++	±	+	±	+++	−	++	++	++	−	+	+
A. ligtu	+++	+++	++	+	+++	+++	+++	++	+++	+++	+++	+
A. pelegrina	+++	±	±	++	+++	−	±	+++	+++	−	+	+++
A. philippii	+++	±	±	±	+++	−	±	±	+++	−	±	±
A. psittacina 22622	++	+	+	++	++	+	++	++	++	+	+	++
A. psittacina 21378	+	++	++	+	+	++	+++	++	+	++	++	++
A. pulchra 22623	+++	±	++	++	++++	±	+++	++	+++	±	++	++
A. revoluta	+++	++	+++	±	+++	+	+++	−	+++	+	+++	±
A. versicolor	+++	++++	+++	+	++++	++++	+++	++	+++	0	++	+
A. violacea	++	±	++	++	+++	+	+++	++	+++	±	++	++
A. taxa 22624	++	+	±	+	+++	++	+	++	+++	+	+	+
22695	+++	++	+++	++	+++	+++	+++	++	+++	+	++	+
22616	+++	±	++	±	+++	±	++	±	+++	±	++	±
"Aalsmeer 4420"	++++	++	++	±	++++	++	++	±	+++	++	++	±
Bomarea carderi	0	−	+	0	0	−	++	0	0	−	+	0
B. edulis	+	±	++	0	−	−	++	0	±	±	+	0
B. spec. 25/6	±	±	+	0	−	−	−	0	−	−	±	0
Erythronium dens-canis	++	+++	+++	++	++	+++	++	++	+	++	+	+
E. revolutum	+++	+++	+++	++	+++	+++	+++	++	++	+++	++	+
E. tolumnense	+++	++	++	+++	+++	++	++	++	++	+	+	++
E. cv. kondo	0	+++	++	+	+++	+++	+++	++	0	+++	++	+
E. cv. Pagode	++	++	++	−	+++	+++	+++	+	++	++	++	+
Fritillaria nigra	−	±	−	−	−	−	−	−	−	−	−	−
Gagea fistulosa	−	−	−	±	−	−	−	−	−	−	−	−
G. lutea 23418	±	+	+	−	−	−	−	−	±	±	±	−
G. lutea 19854	±	+	+	−	−	+	−	−	±	±	±	−
G. lutea 5956	±	+	+	−	−	−	−	0	−	−	−	0
G. lutea 19617	+	+	+	−	−	−	−	−	±	+	+	−
G. minima	+	+	+	−	−	−	−	−	0	+	±	−
G. saxatilis	±	−	0	±	−	−	−	−	−	−	−	−
Tulipa acuminata	++	++	++	++	+++	0	+++	+++	++	0	++	++
T. batalinii	++	++	+++	+	+++	++	+++	+	+++	+++	+++	+
T. biflora	++	++	+++	±	++	++	+++	−	++	++	+	−
T. celsiana	+++	+++	+++	±	++	++	+++	−	+++	+++	+++	±
T. chrysantha	++	++	++	±	++	++	++	−	+	+	+	−
T. clusiana	++	++	++	±	++	+++	+++	−	++	+++	+++	±
T. didieri	++	++	++	+++	+++	++	+++	+++	+++	+	++	+++
T. eichleri	±	−	±	±	+	−	−	−	−	−	+	+
T. kolpakowskiana	+	+	±	−	0	0	0	0	++	++	+	−
T. mauritiana	+++	++	+++	++	+++	0	+++	++	+++	++	+++	++
T. turkestanica	+	++	++	±	±	++	++	−	+	++	++	±
T. urumiensis	++	+++	+++	±	+++	+++	+++	−	+	++	+++	±
T. whittallii	++	+++	+++	+	+++	+++	+++	+	++	+++	+++	+
Allium cepa	±	−	±	−	−	−	−	−	−	−	−	−
Dioscorea cf. *hispida*	−	±	−	0	−	−	−	0	−	−	−	0
Smilacina racemosa	±	−	−	±	−	−	−	−	−	−	−	−

* For documentation numbers see Table 1; numbers only given if the same taxon was investigated more than once.

mined. Table 1 reports all plants studied for the presence of tuliposides and Table 2 includes all taxa for which the presence of tuliposides was established by at least one of the above analytical methods.

Table 2 needs some explanation and comments. Under our conditions, tuliposides are detectable if their concentration in fresh plants is above 0·01%. Negative findings imply that tuliposides are absent or present in trace amounts only (less than 0·01%). GLC is of course by far the most discriminating and most sensitive of our analytical methods. By using amounts of extracts corresponding to 5000, 2500 and 15 μg of fresh plant for PC, TLC and GLC analysis respectively, approximately the same limit of detectability of tuliposides was obtained. Nevertheless, consultation of Table 2 demonstrates clearly that GLC is still the more sensitive method (e.g. *Bomarea*, *Gagea*). The amounts of tuliposides reported in Table 2 are based on quantitative determinations (PC, GLC) and on estimation of spot intensities (TLC).

contain detectable amounts of these constituents

Tuliposide B											
GLC				PC				TLC			
F	L	S	B	F	L	S	B	F	L	S	B
–	–	–	–	–	–	–	–	–	–	–	–
–	–	–	–	–	–	–	–	–	–	–	–
–	–	–	–	–	–	–	–	–	–	–	–
–	–	–	–	–	–	–	–	–	–	–	–
–	–	–	–	–	–	–	–	–	–	–	–
–	–	–	–	–	–	–	–	–	–	–	–
–	–	–	–	–	–	–	–	–	–	–	–
–	–	–	–	–	–	–	–	–	–	–	–
–	–	–	–	–	–	–	–	–	–	–	–
–	–	–	–	–	–	–	–	–	–	–	–
–	–	–	–	–	–	–	–	–	–	–	–
–	–	–	–	–	–	–	–	–	–	–	–
–	–	–	–	–	–	–	–	–	–	–	–
–	–	–	–	–	–	–	–	–	0	–	–
–	–	–	–	–	–	–	–	–	–	–	–
–	–	–	–	–	–	–	–	–	–	–	–
–	–	–	–	–	–	–	–	–	–	–	–
–	–	–	–	–	–	–	–	–	–	–	–
–	–	–	–	–	–	–	–	–	–	–	–
0	–	–	0	0	–	–	0	0	–	–	0
–	–	–	0	–	–	–	0	–	–	–	0
–	–	–	0	–	–	–	0	–	–	–	0
+	+ +	+ +	±	+ +	+ +	+ +	–	+	+	+	±
+ +	±	±	+ +	+	–	–	+	+	±	±	+
+ +	+	±	±	+ +	+	–	–	+	±	±	–
0	±	±	±	+ +	–	–	–	0	–	–	–
+	+	–	–	–	–	–	–	+	+	–	–
–	–	–	–	–	–	–	–	–	–	–	–
–	–	–	–	–	–	–	–	–	–	–	–
–	–	–	–	–	–	–	–	–	–	–	–
–	–	–	–	–	–	–	–	–	–	–	–
–	–	–	–	–	–	–	0	–	–	–	0
–	–	–	–	–	–	–	–	–	–	–	–
–	–	–	–	–	–	–	–	0	–	–	–
–	–	0	–	–	–	–	–	–	–	–	–
+ +	+ +	+	–	+	0	+ + +	–	+ +	0	+	–
+ +	+ + +	+ +	–	+ + +	+ + +	+ +	–	+ + +	+ + +	+ +	–
+ +	+ + +	+ +	–	+	+ + +	+ + +	–	+ +	+ + +	+ +	–
+ +	+ + +	+ +	+	+ +	+ + +	+	±	+ +	+ + +	+	+
+ +	+ + +	+ +	±	+ + +	+ + +	+ +	–	+	+ +	+	–
+ +	+ +	+ +	±	–	+ +	+ +	–	+ + +	+ +	+ +	±
+ +	+ +	+	+	+ + +	+ + +	+ +	+	+ + +	+ +	+	+
+ +	+	+ +	±	–	+ +	+	–	+ +	+	+ +	±
+ +	+ +	+	–	0	0	0	0	+ + +	+ + +	+ + +	–
+ + +	+ + +	+	–	+ + +	0	+	–	+ +	+ +	+	–
+ + +	+ +	+ + +	±	+ +	+ +	+ +	–	+ +	+	+ +	–
+ +	+ +	+ +	±	+ + +	+ + +	+ + +	–	+	+	+	±
+ +	+ +	+	–	+ + +	+ + +	+	–	+ +	+ +	+	–
–	–	–	–	–	–	–	–	–	–	–	–
–	–	–	0	–	–	–	0	–	–	–	0
–	–	–	–	–	–	–	–	–	–	–	–

† F = flower, L = leaf, S = stem, B = subterranean parts (roots, rhizomes, bulbs).
– less than ± 0·01% (not detectable); ± 0·01–0·05%; + >0·05–0·15%; + + >0·15–0·5%; + + + >0·5–1·5%; + + + + more than 1·5% fresh plant tissue; 0 = not analyzed.

In general there is good agreement between the three procedures if low tuliposide levels (<0·05%) are excepted.

An amount of 0·1% and more of tuliposide-A was shown to be present in representatives of the genera *Alstroemeria*, *Bomarea*, *Erythronium*, *Gagea* and *Tulipa* and tuliposide-B could only be detected in species of *Erythronium* and *Tulipa*.

Minor amounts (<0·05%) of tuliposide-A were indicated by GLC for one species of each of the genera *Allium*, *Dioscorea*, *Fritillaria* and *Smilacina*. An absolute proof of the occurrence of tuliposide is not given, of course, by a double peak in the correct position on GLC chromatograms, even if the refined procedures described below (Figs. 1 and 2) are used. In rare instances false positive results might be obtained, especially if very low amounts are present. It seemed appropriate to check some of our results by applying a totally different procedure of detection and estimation of tuliposides. Dr. Beijersbergen from the Bulb Research Centre in Lisse, kindly offered col-

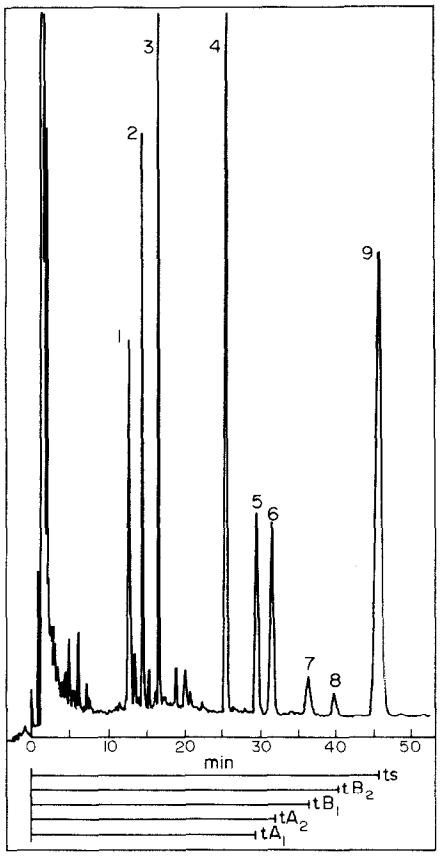

Fig. 1. Capillary Gas Chromatogram of a methanolic extract of the *Erythronium tuolumnense* flowers. Injected 0·1 μl (=15 μg fresh flowers) of TMS-derivatives; SE-30 coated column (22 m × 0·3 mm inner diam). Temperature programming: start: 6 min isotherm at 165°; consequently: 5°/min until 230°; final: 33 min isotherm at 230°. Peaks: 1 = fructose, 2 = α-glucose, 3 = β-glucose, 4 = tetracosan (internal standard), 5 = α-tuliposide-A, 6 = β-tuliposide-A, 7 = α-tuliposide-B, 8 = β-tuliposide-B, 9 = saccharose. Retention times (t): A_1 and A_2 = first and second tuliposide-A peak; B_1 and B_2 = first and second tuliposide-B peak; 2 = saccharose.

laboration; he estimated tuliposide-A contents of some of our extracts by the method described by him [8]. In his procedure tuliposide-A-solutions are adjusted to pH 7·5 and kept for 2 hr at 30°; this induces hydrolysis of the tuliposide and subsequent lactonization of the γ-hydroxy acid. The α-methylene-γ-butyrolactone (tulipalin-A) generated, has a characteristic absorption maximum at 211 nm, which can be used for spectrophotometric estimation. A comparison of the results is given in Table 3. From these results it is clear that tuliposide A occurs in *Bomarea* and *Gagea*. At the same time Table 3 indicates that small amounts of tuliposide-A are indeed present in *Fritillaria nigra* and *Dioscorea hispida*, but presumably not in *Allium cepa* and *Smilacina racemosa*. In conclusion, it may be stated that the more or less general occurrence of tuliposide-A is established for *Alstroemeria*, *Bomarea*, *Erythronium*, *Gagea* and *Tulipa*. It should be stressed once more that minor amounts of tuliposides readily escape observation when applying the methods described here. Our techniques do however, detect tuliposide concentrations which are of practical interest from the viewpoint of their fungitoxic and allergenic actions.

DISCUSSION

Some discrepancies exist between our earlier findings [5] and the present results. These concern the presence of tuliposide-B in *Alstroemeria*, *Calochortus*, *Fritillaria*, *Gagea*, *Lilium*, *Notholirion* and *Tecophilaea* and the occurrence of very small amounts of tuliposide-A in *Calochortus*, *Fritillaria*, *Lilium* and *Notholirion*, which could not be confirmed.

This discrepancy may be explained by the fact that we are now in a better position to distinguish tuliposides from other compounds with a very similar partition ratio. We additionally introduced TLC. Moreover, by using capillary GLC [9], a considerable improvement in separation efficiency has been achieved over conventionally packed column GLC, as used previously [5]. Such a chromatogram is represented by Fig. 1. Double tuliposide peaks are seen due to isomerization of the glucose moieties into α- and β-configurations.

We also observed that extra double peaks sometimes appear on chromatograms of standard sugar mixtures or of plant extracts. The latter most probably are caused by incomplete silylation. By a strange coincidence, these extra peaks have a retention behavior more or less similar to the tuliposides, especially tuliposide-B. Though even on a capillary gas chromatogram such additional double peaks are often hardly distinguishable from genuine tuliposide double peaks, the mutual difference of retention times of the individual peaks forming the pairs (compare Fig. 1) is not the same for tuliposides and peaks due to incomplete silylation. By calculating the difference in retention time for each pair of

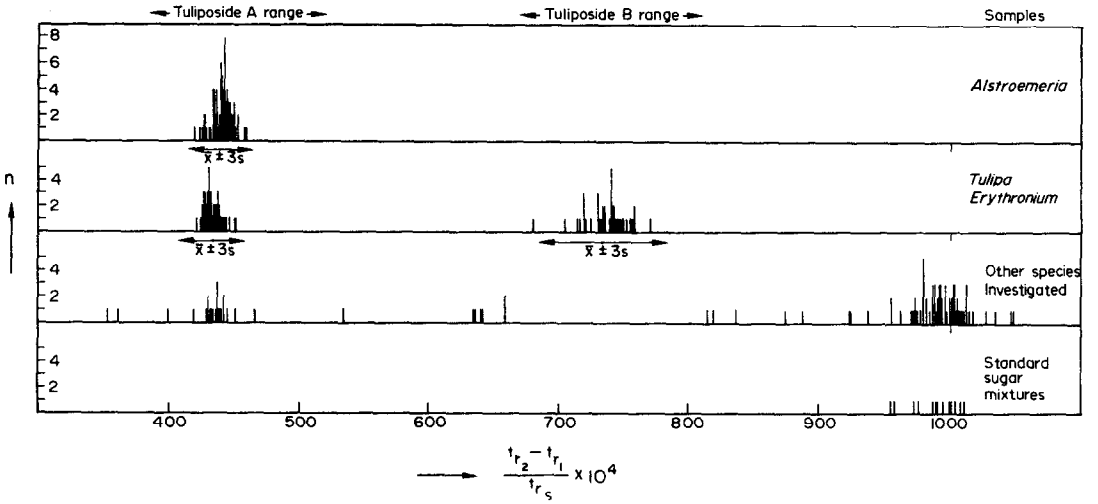

Fig. 2. Statistical analysis of double peaks in the tuliposide ranges of GLC chromatograms. Values = differences in retention times (t_r) for various pairs of peaks in the tuliposide ranges, corrected for chromatogram lengths. $[(t_{r_2} - t_{r_1})/(t_{r_s})]$ as found for the investigated species (multiplied by 10^4).
The values falling within the tuliposide-A range in extracts from "other species investigated" represent: 400 *Smilacina racemosa* (F) Wageningen; 420 *Gagea saxatilis* (F) 22644; 430 *Bomarea edulis* (F) Utrecht; 431 *Smilacina racemosa* (B) Wageningen, *Bomarea edulis* (S) Utrecht; 433 *Bomarea* sp. 25/6 (F); 434 *Gagea lutea* (L) 6947; 435 *Bomarea* sp. 25/6 (L); 437 *Dioscorea hispida* (L) 22638; 438 *Bomarea* sp. 25/6 (S), *Gagea fistulosa* (B) 19869, *Gagea lutea* (L) 19854; 439 *Gagea saxatilis* (B) 22644; 440 *Gagea lutea* (S) 6947; 441 *Gagea lutea* (S) 19854; 442 *Fritillaria nigra* (L) 22642; 443 *Allium cepa* (F) 23421, *Bomarea carderi* (S) Utrecht; 446 *Bomarea edulis* (L) Utrecht; 452 *Gagea lutea* (F) 19854; 467 *Allium cepa* (S) 23421. Of these, *Allium cepa* and *Smilacina racemosa* are excluded as tuliposide-A containing samples by lactone–UV–absorption (Table 3) whereas the others are confirmed.

peaks in the given chromatogram areas and correcting for chromatogram length by dividing with the retention time of an arbitrarily chosen standard (sucrose), one gets a series of values which make statistical interpretation of double peaks possible.

The procedure is illustrated by Fig. 2. *Tulipa* and *Erythronium*, containing large amounts of tuliposides A and B and never causing difficulties in the interpretation of double peaks, are used for reference. Figure 2 shows that tuliposide values for *Tulipa* and *Erythronium* are clearly randomly distributed. Such a Gaussian distribution is characterized by standard deviation and mean value. The distribution of tuliposide-A values obtained with *Alstroemeria* taxa is statistically indistinguishable from the corresponding *Tulipa-Erythronium* distribution. In Gaussian distributions 99·6% of all values fall within a range of the mean ± three times the standard deviation (s). In practice all values outside this range can be considered as not belonging to the distribution concerned. For this reason many of the values found for species from other than the three genera mentioned cannot be considered as indicating presence of either tuliposide A or B. Many values (plant extracts, sugar mixtures) are rather far beyond the tuliposide-B range. On the other hand a number of values obtained with plant extracts distinctly fall within the tuliposide-A range (Fig. 2). For these plants, the probability of them containing tuliposide-A is very high indeed.

Table 3. Detection and estimation of tuliposide-A in selected plant samples by spectrophotometric lactone determination [7] and by the GLC procedure

Taxon	Organ*	Lactone determination	GLC
Allium cepa	F	0	±
Alstroemeria hookeri	L	+++	+++
Alstroemeria revoluta	L	++	++
Bomarea edulis	S	++	++
Dioscorea hispida	L	+	±
Erythronium cv. kondo	L	+++	+++
Fritillaria nigra	L	+	±
Gagea lutea (19854)	L	+	+
Smilacina racemosa	F	0	±
Tulipa batalinii	L	+++	++

* See footnotes in Table 2 for keys.

The possibility that trace amounts of tuliposides do occur in some other taxa is not ruled out. Our present findings with *Fritillaria nigra*, our former findings [5], as well as the observation of Rossetti and Suria [10] with *Lilium candidum* bulbs indicate indeed that minor amounts of tuliposide-like constituents are more widespread in Liliiflorae. The amounts, however, seem to be too small in these taxa for having any significance as producers of post-inhibitins [11] or as causative agents of allergic contact dermatitis.

From the medical point of view it has become clear that all species of *Alstroemeria* are potentially allergenic; their increased commercial production as ornamental plants may cause in future similar skin troubles as are known from workers in tulip cultures. The first reports of the occurrence of an *Alstroemeria* allergy in Aalsmeer have actually already reached the Dermatological Department of the Free University of Amsterdam [12].

With regard to plant systematics, the present investigation definitely demonstrated that Alstroemeriaceae (many species of *Alstroemeria*, two species of *Bomarea*) share the tuliposide-character with a number of Liliaceae *sensu strictu* (= Lilioideae). This agrees very well with the proposals of Buxbaum [13] and especially of Huber [6] concerning relationships of this epigynous (or pseudo-epignynous: Buxbaum) taxon. According to Buxbaum, Alstroemeriaceae evolved from liliaceous (especially *Lilium*) ancestors. Huber, however, believes that such a derivation is impossible because Alstroemeriaceae share many characters with Colchicaceae and others with Liliaceae and at the same time possess characters which are more primitive than the corresponding characters in *Lilium* and in Colchichaceae. Therefore he assumes divergent evolution of Liliaceae *s.s.*, Alstroemeriaceae and Colchicaceae from common ancestors.

If tuliposides are biosynthetically related to γ-methyleneglutamic acid, which is present in considerable amounts in many Liliaceae *s.s.* (= Lilioideae) and occurs sporadically in other liliaceous taxa [14], the distribution of tuliposides would be understandable. A metabolic pattern which allows for the origin of the tuliposides made selection for their production and accumulation possible. A defense mechanism based on tuliposides was acquired by two diverging evolutionary lines, *Gagea-Tulipa-Erythronium* and *Bomarea-Alstroemeria*, but not by the lines resulting in present day Colchicaceae, *Calchortus*, *Tricyrtis* and *Lilium-Fritillaria*. Other mechanisms of defense might have been more appropriate or more essential under the conditions of their divergent evolution. The general presence of colchicine and related alkaloids in Colchicaceae is highly suggestive in this respect.

It seems worthwhile to mention that antibiotically active mechanisms resembling the "tuliposides → lactone-system" in several respects, also occur in *Narthecium* (Melanthiaceae) [15] and in many Ranunculaceae [16] (ranunculin → protoanemonin).

According to Huber [6] Trilliaceae (including *Medeola* and *Scoliopus*) are on the borderline between Dioscoreoid and Colchicoid Liliiflorae. Therefore we were anxious to investigate several members of this taxon. *Medeola virginiana*, *Scoliopus bigelovii*, *Paris quadrifolia* and six species of *Trillium* (Table 1) were available to us. None of them accumulates tuliposides.

EXPERIMENTAL

Preparation of extracts. A known amount of fresh plant tissue was cut into small pieces. A suitable volume of MeOH was added and the whole was homogenized in a blender (20000 rpm; 5 min). After centrifugation (2000 rpm; 15 min) the supernatant was collected and the residues re-extracted with the same amount of MeOH. After centrifugation (30 min) the two supernatants were combined and recentrifuged (15 min). The vol. of transparent MeOH soln was ascertained. Extracts were stored in the dark at 4° until they could be analyzed. Tuliposides remain unaltered during several months in extracts prepared and stored as described. The procedure described removes 95% of the extractable tuliposides present in a plant organ.

Paper chromatography was by descent. A vol. of extract corresponding to 5 mg of fresh plant tissue was deposited on Whatman no. 3 paper, which was developed in *n*-BuOH–Me_2CO–H_2O (5:1:2). Tuliposides were located on chromatograms by an ammonical $AgNO_3$ spray [17].

Thin Layer Chromatography. An amount of extract corresponding to 2·5 mg of fresh plant material was deposited on Kieselgel-Al-sheets (Merck). Solvent system: isopropanol 65%–EtOAc (7:13). Tuliposides were detected by spraying with a solution of diphenylamine and aniline in phosphoric acid and acetone [18] resulting in blue spots.

Capillary gas chromatography (Fig. 1). An amount of plant extract corresponding to 75 mg fresh parts was completely dried and dissolved in 0·3 ml dimethylformamide and 0·2 ml *bis*-(trimethylsilyl)-acetamide (TMS). After silylation 0·1 μl. (corresponding to 15 μg of fresh plant material) of this mixture was injected for separation on a SE-30 coated capillary column prepared according to Slob and Luteyn [9] (see Fig. 1 for further details).

Determination of tuliposide contents. Densitometric procedure (Joyce–Loebl chromoscan) for PC and digital–analog conversion and subsequent computer calculation for GLC. With TLC plates the intensities of spot colours were visually compared only. Because each chromatogram was prepared with a known amount of fresh plant tissue, contents could easily be calculated from the amount of tuliposides present on a given chromatogram. As no pure tuliposides were available for calibration, glucose was used for this purpose.

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REFERENCES

1. Tschesche, R., Kämmerer, F. J., Wulff, G. and Schönbeck, F. (1968) *Tetrahedron Letters* **6,** 701; *idem* (1969) *Chem. Ber.* **102,** 2057.
2. Beijersbergen, J. C. M. (1969) Thesis, University of Leiden; Beijersbergen, J. C. M. and Limmers, C. B. G. (1972) *Physiol. Plant Pathol.* **2,** 265.
3. Schönbeck, F. (1967) *Phytopathol. Z.* **59,** 205; Schönbeck, F. and Schroeder, C. (1972) *Physiol. Plant Pathol.* **2,** 91; Schroeder, C. (1972) *Phytopathol. Z.* **74,** 175; *Z. Pflanzenkrankh. Pflanzenschutz* **79,** 1.
4. Verspyck Mijnssen, G. A. W. (1968) Thesis, Univ. of Leiden.
5. Slob, A. (1973) *Phytochemistry* **12,** 811.
6. Huber, H. (1969) *Mitt. Bot. München* **8,** 219.
7. Melchior, H. (1964) Liliales in: Engler's *Syllabus der Pflanzenfamilien* 12th edn., Vol. 2. Gebrüder Borntraeger, Berlin.
8. Beijersbergen, J. C. M. (1972) *Rec. Trav. Chim. Pays-Bas* **91,** 1193.
9. Slob, A. and Luteyn, L. (1974) *Pharm. Weekblad* **109,** 901.
10. Rossetti, V. and Suria, M. (1972) *Phytochemistry* **11,** 859.
11. Ingham, J. L. (1973) *Phytopathol. Z.* **78,** 314.
12. Van Ketel, W. G., dermatologist, Academical Hospital, Free University of Amsterdam; personal communication.
13. Buxbaum F. (1951) *Phytomorphology* **1,** 170; (1954) *Österr. Botan. Z.* **101,** 337.
14. Fowden, L. and Steward, F. C. (1957) *Ann. Botany* **21,** 53.
15. Tschesche, R. and Hoppe, H. J. (1971) *Chem. Ber.* **104,** 3573.
16. Ruijgrok, H. W. L. (1966) in *Comparative Phytochemistry* (Swain, T., ed.), p. 175. Academic Press, London.
17. Merck, E. (1959) *Anfärbereagenzien für die Papierchromatographie*; reagent 16b, Merck, Darmstadt.
18. Stahl, E. (1967) *Dünnschichtchromatographie*, 2nd. edn. Springer-Verlag, Berlin.