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Sesquiterpene lactones

Part XXII. Sesquiterpene lactones in Species of the Genus *Chrysanthemum*

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ABSTRACT

A method of effective study on sesquiterpeine lactones in unknown plant material was worked out.
This method allows the choice of species with high sesquiterpene lactones content and interesting chemical composition.
Studies were carried out on 14 species of *Chrysanthemum* s.l.. The method of elementary appreciation consisted in: estimation of the bitter principle index (assayed by the modified Ph.P.IV. method), quantitative analysis of lactone fractions and chromatographical examination.
Quantitative analysis of sesquiterpene lactones in plant material was carried out by the IR spectroscopy method specially adapted for this purpose.
Chromatographic examinations were carried out by the thin-layer technique on silica gel. Chromatograms of lactone fractions were developed with 6 selective reagents.
The correlation between the bitter principle index and quantitative content of sesquiterpene lactones was ascertained.
Bisides great qualitative also quantitative differences were observed between 14 investigated species from *Chrysanthemum* s.l.

INTRODUCTION

The systematic classification of plants of the genus *Chrysanthemum* L. has occasioned numerous controversies, mainly on account of the wide morphological variability of the particular systematic units. This variability in due both to the influence of the environment and to the

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tendency to fromation of polymorphic species and hybrids (S z e n n i k o w, 1966). Therefore there exist in the world literature numerous synonymic names and new systematic classifications are suggested for the genus *Chrysanthemum*. This concerns particularly the grouping of species in sections and the establishment, beside the genus *Chrysanthemum*, of those of *Pyrethrum*, *Tanacetum* and others as equal in rank (U.S.S.R. F l o r a, 1961; S z a f e r et al., 1953).

Table 1

Systematic division of the investigated species from the genus *Chrysanthemum* s.l.

Species	Genus	Section	Genus	Section
	H o f f m a n n (1897)		ZSRR Flora (1961)	
Chr. carinatum Schousb.	*Chrysanthemum* L.	*Ismelia* Cass.	*Chrysanthemum* L.	*Ismelia* (Cass.) DC.
Chr. anethifolium Brouss.	*Chrysanthemum* L.	*Argyranthemum* Webb.	—	—
Chr. leucanthemum L.	*Chrysanthemum* L.	*Pyrethrum* Grtn.	*Leucanthemum* Mill.	—
Chr. lacustre Brot.	*Chrysanthemum* L.	*Pyrethrum* Grtn.	—	—
Chr. parthenium (L.) Bernh.	*Chrysanthemum* L.	*Pyrethrum* Grtn.	*Pyrethrum* Zinn.	*Parthenium* (Brig.) Tzvel.
Chr. parthenifolium Pers.	*Chrysanthemum* L.	*Pyrethrum* Grtn.	*Pyrethrum* Zinn.	*Parthenium* (Briq.) Tzvel.
Chr. prealtum Vent.	*Chrysanthemum* L.	*Pyrethrum* Grtn.	*Pyrethrum* Zinn.	*Parthenium* (Briq.) Tzvel.
Chr. corymbosum L.	*Chrysanthemum* L.	*Pyrethrum* Grtn.	*Pyrethrum* Zinn.	*Pyrethrum*
Chr. subcorymbosum Schur.	*Chrysanthemum* L.	*Pyrethrum* Grtn.	—	—
Chr. indicum L.	*Chrysanthemum* L.	*Pyrethrum* Grtn.	*Dendranthema* (DC) Des Moul.	*Dendranthema* (DC) Des Moul.
Chr. balsamita L.	*Chrysanthemum* L.	*Pyrethrum* Grtn.	*Pyrethrum* Zinn.	*Balsamita* (Mill.) DC.
Chr. serotinum L.	*Chrysanthemum* L.	*Pyrethrum* Grtn.	*Leucanthemella* Tzvel.	—
Chr. macrophyllum W. et K.	*Chrysanthemum* L.	*Gymnocline* Cass.	*Pyrethrum* Zinn.	*Gymnocline* (Cass.) DC.
Chr. vulgare (L.) Bernh.	*Chrysanthemum* L.	*Tanacetum* L.	*Tanacetum* L.	*Tanacetum*

Correct classification of plants of the genus *Chrysanthemum* exhibiting an exceptionally high variability may be rendered easier by taking into account in taxonomic studies, beside the differences in the morphological structure, also other characteristics of these plants.

Sesquiterpene lactones are considered to be an important chemo-

taxonomic character of systematic units of the family *Compositae*. Their knowledge in the particular plants should facilitate correct classification of the genus *Chrysanthemum* s.l. (Hegnauer 1964; Herout 1971, 1973).

It results from the review of world literature that the species of the genus *Chrysanthemum* s.l. synthesize various types of sesquiterpene lactones (e.g. Bańkowskij 1969; Romo et al., 1970; Doskotch 1971).

The present investigation was undertaken to establish the ability of lactone sesquiterpene synthesis and accumulation in 14 *Chrysanthemum* s.l. species. It was assumed that comparison of chromatographic results, bitter principle indexes and sesquiterpene lactones content would reveal the species containing large quantities of the latter substances.

MATERIAL AND METHODS

The investigations concerned 14 plants species belonging to the genus *Chrysanthemum* sensu lato.

Material for the studies consisted of above ground pats dried at 40°C, collected in the period of flowering. The plants were cultivated in the Pharmacognostic Garden, Institute of Biology and Pharmacy, Academy of Medicine, Poznań.

The species examined belonged to 6 sections according to Hoffmann (1897). Table 1. shows also the classification of these species suggested by the U.S.S.R. Flora (1961).

DETERMINATION OF THE BITTER PRINCIPLE INDEX

Most sesquiterpene lactones are characterized by a very bitter taste. The value of the bitter principle index may, therefore, supply preliminary information on the accumulation of these substances in the particular species.

The bitter principle index was determined for 14 species of *Chrysanthemum* s.l. by the taste method after Polish Pharmacopeia IV (1970).

A modification was introduced consisting in the preparation of aqueous extracts at 60°C and not at 100°C as required by Ph. P. IV. (1970). This was done since it was observed that at higher temperature the lactone sesquiterpenes are partly decomposed, as indicated by a marked decrease in the bitter principle index (Table 2.).

As demonstrated by the studies, the bitter principle index varies from 80 for *Chrysanthemum macrophyllum* to 1 for *Chrysanthemum leucanthemum* and *Chrysanthemum lacustre*.

The species with the highest index is classified to the section *Gym-*

nocline Cass., and the one with the lowest index to the section *Pyrethrum* Grtn.

Notheworthy are the wide differences in the bitter principle index in the species classified to the section *Pyrethrum* Grtn. (65 — 1). However, if these species belonging to the section *Pyrethrum* Grtn. according to Hoffmann (1897) would be reclassified according to the U.S.S.R. Flora (1961), they would appear in several sections of the genus *Pyrethrum* Zinn. or even in completely different genera. Then the correlation would become apparent between the appartenance to a given section and the value of the bitter principle index. It namely appears that the highest bitter principle index is characteristic for species of the section *Balsamita* (Mill.) DC and *Parthenium* (Briq.) Tzvel. of the genus *Pyrethrum* Zinn. On the other hand, the lowest index is found in species classified by the U.S.S.R. Flora (1961) to the genus *Leucanthemum* Mill. and the section *Pyrethrum* of the genus *Pyrethrum* Zinn. (Table 2.).

ISOLATION OF LACTONE FRACTIONS

In the method of sesquiterpene lactones isolation from the plant material, applied in the present study, a chloroform extract is obtained from the aqueous extract purified with lead acetate.

This extract was denoted as "the lactone fraction" (Drożdż and Piotrowski, 1973).

10,0 g of dried above ground parts were comminuted in a mill and thrice extracted with 200 ml portions of methanol at 40°C. The extracts were combined and concentrated under vacuum to a small volume. To the remainder distilled water was added and the remaining methanol was distilled off once more under vacuum. To the aqueous extract obtained in this way (vol. ca 50 ml) a saturated lead acetate solution was added until encumbering substances were completely precipitated. Then the extract was left to stand for 1 h at room temperature and centrifuged. The clear aqueous solution from over the sediment was three times extracted with chloroform, each time with 100 ml of this solvent. The chloroform layers were separated from the aqueous ones, combined, dried with anhydrous sodium sulphate, filtrated and concentrated to dryness. The chloroform extract thus obtained had the consistence of a brown very thick syrup and constituted the lactone fraction for further investigation.

The yields of the obtained lactone fractions from the 14 examined species are shown in Table 2.

The investigations demonstrated that species of the section *Pyrethrum* Grtn. exhibit the highest yield of lactone fraction and next came the species of the section *Gymnocline* Cass. and *Tanacetum* L. The

lowest yield was obtained from species classified to the section *Pyrethrum* Grtn. and those of section *Ismelia* Cass. and *Argyranthemum* Webb.

If the division according to the U.S.S.R. Flora (1961) would be, however, applied to the species comprised in the section *Pyrethrum* Grtn., two species with highest yield of lactone fraction — *Chr. parthenium* (L.) Bernh. and *Chr. balsamita* L. — would appear in the sections *Parthenium* (Briq.) Tzvel. and *Balsamita* (Mill.) DC. of the genus *Pyrethrum* Zinn., whereas the species with lowest would be classified to the section *Pyrethrum* Zinn. of the genus *Pyrethrum* Zinn.

Moreover, a high correlation was found between the lactone fractions content and the bitter principle index in the examined species of the genus *Chrysanthemum* s.l..

QUANTITATIVE DETERMINATION OF SESQUITERPENE LACTONES CONTENT IN RAW PLANT MATERIAL BY THE METHOD OF INFRARED SPECTROSCOPY

Sesquiterpene lactones content was determined by the method of infrared spectroscopy and expressed as parthenolide and tanacetin — lactones occurring in species of the genus *Chrysanthemum* s.l. (Samek et al., 1973; Souček et al., 1961).

For the determinations lactone fractions isolated from 10,0 g of dried green mass of 14 species of the genus *Chrysanthemum* s.l. were used.

A detailed description of the procedure in preparation of standard lactone solutions and fractions for quantitative measurements as well as the pertinent technical problems of measurement are described in other publications (Błoszyk, 1975; Błoszyk et al., 1977).

Absorption coefficients (K) were determined on the basis of Beer's law (Alpert et al., 1974) for the standard lactones; for parthenolide K = 227 and for tanacetin K = 119.

Further sesquiterpene lactones content was determined in extracts from 10,0 g dried plants of 14 species of the genus *Chrysanthemum* s.l. The results are listed in table 2 converted to 100,0 g of dry material.

There was no difficulty in determination of the sesquiterpene lactones content in the green parts of *Chrysanthemum parthenium* (L.) Bernh. and *Chrysanthemum vulgare* (L). Bernh since the corresponding standard substances — parthenolide and tanacetin — were available, extracted from the aerial parts of these species.

In the material of the remaining 12 species sesquiterpene lactones were determined in conversion to parthenolide and tanacetin. It was realized that this will affect the results, however, it was assumed that data obtained will have a comparable value and will allow to designate species with a high sesquiterpene content.

The analyses showed that sesquiterpene lactone content in the species examined varied in the limits of 1 — 80, notwithstanding the kind of standard used (parthenolide or tanacetin). The results converted to parthenolide gave by one half lower values than when to tanacetin (Table 2).

Table 2

Values of the bitter principle index, yield of lactone fractions and percentage of sesquiterpene lactones in dry material from 14 species of *Chrysanthemum* s.l.

Species	Section after Hoffmann (1897)	Bitter principle index		Yield of lactone fractions (g)	Percentage of sesquiterpene lactones in dry material (count over)	
		60°C	100°C		tanacetin %	parthenolide %
Chr. parthenium (L.) Bernh.	*Pyrethrum* Grtn.	65	30	2.22	1.58	0.87
Chr. balsamita L.	,,	63	50	1.90	0.93	0.50
Chr. macrophyllum W. et K.	*Gymnocline* Cass.	80	63	0.97	0.44	0.22
Chr. vulgare (L.) Bernh.	*Tanacetum* L.	60	—	0.88	0.33	0.16
Chr. prealtum Vent.	*Pyrethrum* Grtn.	55	—	0.53	0.34	0.18
Chr. indicum L.	,,	10	—	0.52	0.15	0.08
Chr. serotinum L.	,,	30	—	0.31	0.07	0.04
Chr. leucanthemum L.	,,	1	—	0.26	0.06	0.03
Chr. lacustre Brot.	,,	1	—	0.23	0.05	0.03
Chr. parthenifolium Pers.	,,	50	—	0.16	0.05	0.03
Chr. carinatum Schousb.	*Ismelia* Cass.	20	—	0.14	0.04	0.02
Chr. anethifolium Brouss.	*Argyranthemum* Webb.	20	—	0.12	0.03	0.02
Chr. subcorymbosum Schur.	*Pyrethrum* Grtn.	20	—	0.10	0.02	0.01
Chr. corymbosum L.	,,	5	—	0.04	0.02	0.01

Quantitative investigations revealed that the lowest sesquiterpene content occurs in species showing the lowest bitter principle index and giving the lowest yield of lactone fractions (*Chr. corymbosum* L., *Chr. subcorymbosum* Schur. from the *Pyrethrum* Grtn. section). Similarly, the highest lactone content was found in species with the highest bitter principle index and high yield of lactone fractions — (*Chr. parthenium* (L.) Bernh., *Chr. prealtum* Vent., *Chr. balsamita* L. from the section *Pyrethrum* Grtn. and *Chr. macrophyllum* W. et K. from the *Gymnocline* Cass. section).

CHROMATOGRAPHIC ANALYSIS

Thin layer chromatography on glass silica gel-coated plates with an addition of slow-associated gypsum (D r o ż d ż, 1967) was used for analysis of the composition of lactone fractions. At the starting points 200 μg lactone fraction was placed and the chromatograms were developed with 3 solvent systems:

I — chloroform — acetone (9:1)
II — benzene — acetone (9:1)
III — ethyl ether

The colour of the developed and dried chromatograms were detected by means of selective reagents.

1) resorcin solution with concentrated H_3PO_4 added
2) fructose solution with concentrated H_2SO_4 added
3) $FeCl_3$ solution with concentrated H_3PO_4 and H_2SO_4 added (D r o ż d ż and B ł o s z y k, 1977).

Parthenolide isolated by S o u č e k et al., (1961) from *Chrysanthemum parthenium* (L.) Bernh. and erivanin isolated from *Chrysanthemum balsamita* L. by S a m e k et al., (1975) were used as standards.

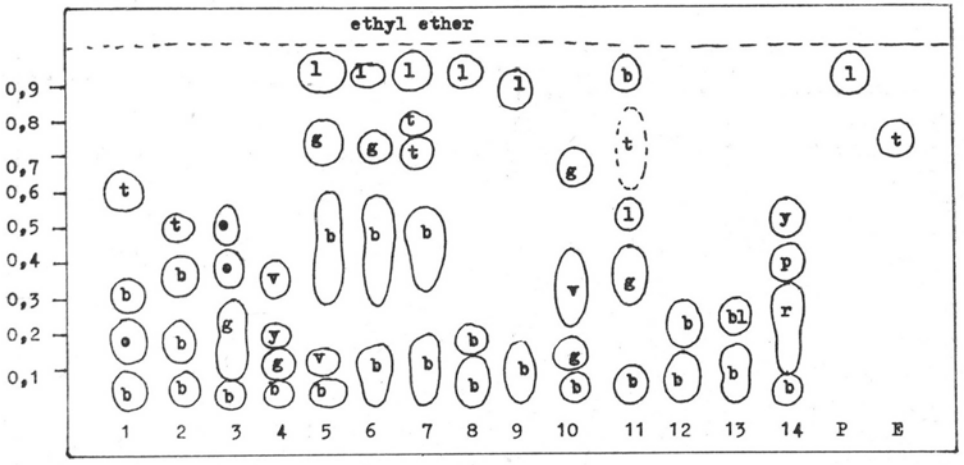

Fig. 1. TL chromatogram of lactone fractions from 14 species of *Chrysanthemum* s.l. obtained by using as detector reagent — 0,05 % $FeCl_3$ mixed with 62,5 parts of concentrated H_3PO_4 and 37,5 parts of concentrated H_2SO_4

Abbreviations: 1 — *Chr. carinatum*, 2 — *Chr. anethifolium*, 3 — *Chr. leucanthemum*, 4 — *Chr. lacustre*, 5 — *Chr. parthenium*, 6 — *Chr. parthenifolium*, 7 — *Chr. prealtum*, 8 — *Chr. corymbosum*, 10 — *Chr. indicum*, 11 — *Chr. balsamita*, 12 — *Chr. serotinum*, 13 — *Chr. macrophyllum*, 14 — *Chr. vulgare*, P — parthenolide, E — erivanin. Colorations of spots: y — yellow, o — orange, p — pink, r — red, ch — cherry, b — brown, bl — blue, l — lilac, t — turquoise, v — violet, g —green, gr — grey.

Comparison of the chromatograms (Figs 1, 2, 3) suggested that the particular *Chrysanthemum* s.l. species exhibit wide differences in the

composition of the lactone fractions. A characteristic feature of most of the *Chrysanthemum* s.l. species seems to be the ability to synthesize numerous different sesquiterpene lactones.

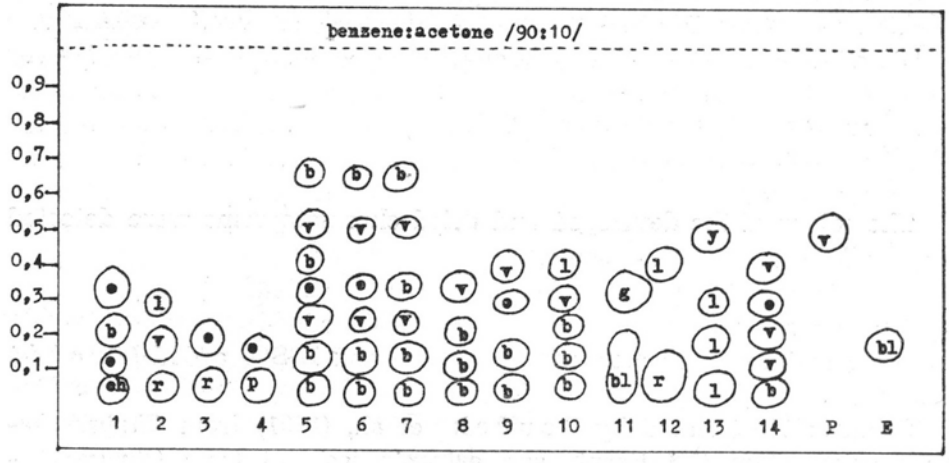

Fig. 2. TL chromatogram of lactone fractions from 14 species of *Chrystanthemum* s.l. obtained by using as detector reagent — 1% fructose mixed with 2% H_2SO_4. For abbreviations — see Fig. 1

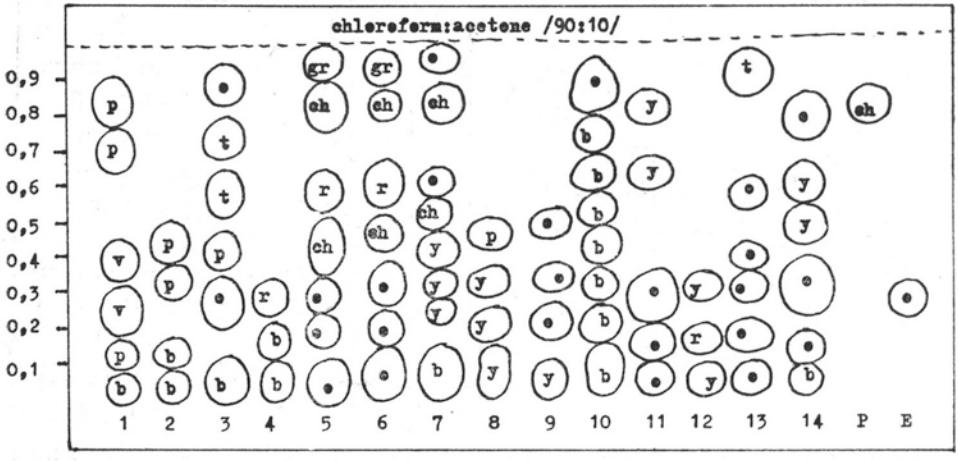

Fig. 3. TL chromatogram of lactone fractions from 14 species of *Chrysanthemum* s.l. obtained by using as detector reagent — 1% resorcin mixed with 5% H_3PO_4. For abbreviations — see Fig. 1

Most closely similar were the chromatograms of *Chrysanthemum parthenium* (L.) Bernh., *Chr. prealtum* Vent. and *Chrysanthemum parthenifolium* Pers. this confirming their close relationship (U.S.S.R. Flora, 1961) and the possibility of the presence of parthenolide in them.

On the other hand, wide differences were observed in the composition of the lactone fractions in the species examined belonging to different sections of the genus *Chrysanthemum* s.l. Further reaching conclusions could not be drawn since the available standard substances were limited to only two lactones isolated from the genus *Chrysanthemum* — parthenolide and erivanin. A definitive discussion concerning the spots on the chromatograms will only be possible when chromatographically homogeneous compounds will be isolated from the studied *Chrysanthemum* s.l. species and identified.

RESULTS AND CONCLUSIONS

Comparison of the bitter principle indexes, lactone fraction yields and of the percentual sesquiterpene lactones content as well as of chromatograms with colour developed by selective reagents supplied much information as regards the occurence of sesquiterpene lactones in 14 species of *Chrysanthemum* sensu lato.

It was found that the particular species show a different ability of synthesizing and accumulating these lactones.

A high correlation of results was obtained. The species showing a high bitter principle index gave at the same time the highest yield of lactone fractions with the highest per cent of sesquiterpene lactones.

Lactones were most abundant in the species of the section *Gymnocline (Chr. macrophyllum), Tanacetum (Chr. vulgare), Balsamita (Chr. balsamita)* and *Parthenium (Chr. parthenium)* which, according to the authors of the U.S.S.R. Flora (1961), belong to the genus *Pyrethrum* Zinn. The lowest sesquiterpene lactones content was noted in species classified in the U.S.S.R. Flora to the section *Ismelia* — the genus *Chrysanthemum* L. *(Chr. carinatum),* the genus *Argyranthemum (Chr. anethifolium),* the section *Pyrethrum* — the genus *Pyrethrum* Zinn. *(Chr. corymbosum).*

Chromatographic check with the use of selective colouring developing reagents demonstrated wide differences in the composition of the lactone fractions isolated from the particular species.

Definitive conclusions concerning the chemical taxonomy of the plants will be only possible after isolation and identification of the sesquiterpene lactones contained in these species.

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Laktony seskwiterpenowe w gatunkach z rodzaju Chrysanthemum s.l.

Streszczenie

Opracowano metodykę postępowania, która pozwoliłaby na świadome poszukiwanie laktonów seskwiterpenowych w obrębie określonej jednostki systematycznej oraz na typowanie gatunków o wysokiej zawartości laktonów seskwiterpenowych i ciekawym składzie chemicznym, szczególnie przydatnych do rozpracowania szczegółowego.

Badania przeprowadzono na modelu 14 gatunków z rodzaju *Chrysanthemum* sensu lato.

Metodyka wstępnej oceny przydatności poszczególnych gatunków obejmowała oznaczenie wysokości wskaźnika goryczy zmodyfikowaną metodą Ph.P.IV., zawartości frakcji laktonowych, ilościowe oznaczenie zawartości laktonów seskwiterpenowych oraz badania chromatograficzne.

Oznaczenie ilościowe zawartości laktonów seskwiterpenowych w materiale roślinnym wykonano adaptując do tego celu metodę spektroskopii w podczerwieni.

Badania chromatograficzne przeprowadzono metodą chromatografii cienkowarstwowej na żelu krzemionkowym, stosując do wywołania laktonów seskwiterpenowych 6 odczynników selektywnie barwiących.

Stwierdzono dużą korelację wysokości wskaźnika goryczy oraz ilościowej zawartości laktonów seskwisterpenowych w badanych surowcach z rodzaju *Chrysanthemum* s.l.

Ponadto stwierdzono duże różnice jakościowe i ilościowe pomiędzy poszczególnymi badanymi gatunkami.