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TLC of selected sesquiterpenoids of the Asteraceae family

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Abstract

Visual chromatography has been employed for a preliminary identification of natural compound of a group of sesquiterpene lactones – *Centaurea bella* Trautv., *C. crocodylium* L., *C. lusitanica* Boiss. et Reuter, *Helenium hoopesii* A. Gray = *Dugaldia hoopesii* (A. Gray) Rydb., *Stizolophus balsamita* (Lam.) Cass., *Zoegea baldschuanica* C. Winkl., germacranes – *Santolina pinnata* Viv. subsp. *neapolitana* (Jord. et Fourr.) Guinea = *S. neapolitana* Jord. et Fourr. A dependence of the colour of the spots, induced by anisaldehyde reagent, on the presence of several substituents in the germacranolide ring – derivatives of partnenolide and salonitenolide – has been identified. The structures of the skeleton in the ring of sesquiterpene lactones in *Helenium hoopesii* can also be established by thin layer chromatography (TLC). The analysis of the chromatograms of extracts from dry and fresh *Centaurea crocodylium* herb has shown significant differences as for the chemical composition. Two sorts of germacranes of *Santolina pinnata* subsp. *neapolitana* display a characteristic colour of the spots.

Keywords: sesquiterpene lactones, Asteraceae, TLC, identification

Introduction

Among natural compounds in the species of family Asteraceae, sesquiterpenoids play an important role. They constitute a valuable chemotaxonomic material and might be also decisive for the medicinal value of the raw material, as in *Chamomillae anthodium*, *Cnici benedicti herba*, *Arnicae anthodium*, *Millefolii herba*, *Chrysanthemi partheni herba*, *Cichorii radix et herba*. A recent study has revealed antibacterial and cytotoxic activities of sesquiterpene lactones [1]. These compounds can be detected through visual chromatography and isolated by simple column chromatography, with silica gel as the adsorbent.

The possibility of identification of several substituents at guaianolides by TLC with concentrated sulphuric acid as the developer had been mentioned earlier [2,3]. Now, visual chromatography of compounds of four other types of sesquiterpenoids has been presented: germacranolides, germacranes, seco-pseudoguaianolides and pseudoguaianolides.

To develop the spots, the sole anisaldehyde reagent, which proved selective enough to rate the colours of the spots of the compounds in question, was used.

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Material and methods

Plant material

The plant materials used for the studies employed aerial parts of *Centaurea bella*, *C. crocodylium*, *C. lusitanica*, *Helenium hoopesii*, *Santolina pinnata* subsp. *neapolitana*, *Stizolophus balsamita*, *Zoegea baldschuanica*, plants cultivated and identified in the garden of Department of Medicinal and Cosmetic Natural Products, University of Medical Sciences in Poznań (Poland), where their voucher specimens are deposited.

Isolation and identification of compounds

The herbs, collected right before blooming (about 450 g each) were extracted with methanol three times. The methanol extracts were inundated with distilled water (ca. 600 cm^3), after the evaporation of the solvent. The water phase was extracted with chloroform. The chloroform extracts, in turn, having been dried with anhydrous sodium sulphate, were used for thin layer chromatography and isolation of compounds. Furthermore, some methanol/water/chloroform extracts were prepared from *Centaurea crocodylium* herb, which had been collected at the same time and from the same plot as the herbs designed to be dried.

The extracts were separated by column chromatography on silica gel (Merck art. 7733). The fractions were subjected to repeated column chromatography on silica gel (Merck art. 7729) and eluted by right mobile phases. The structures of the isolated compounds (Fig. 1) were identified on the basis of 1H NMR, IR and EI mass spectroscopy and by comparing the obtained data with those of the reference compounds or reported data [4-8].

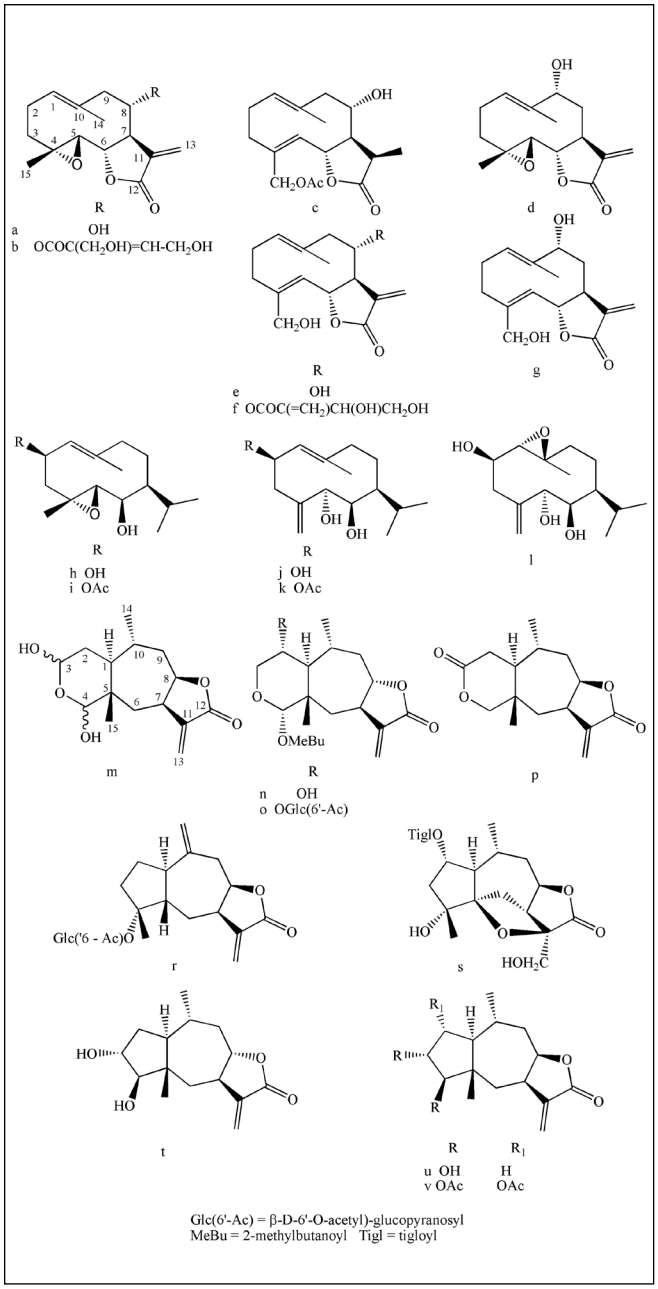

Fig. 1 Chemical structures of: **a** stizolin; **b** stizolicin; **c** cebellin M; **d** 9α-hydroxyparthenolide; **e** salonitenolide; **f** cnicin; **g** stenophyllolide; **h** 4β,5α-epoxy-7αH-germacr-1(10)*E*-ene-2β,6α-diol; **i** 2-acetoxy,4β,5α-epoxy-7αH-germacr-1(10)*E*-ene-6β-ol; **j** 7αH-germacra-1(10)*E*,4(15)-diene-2β,5α,6β-triol; **k** 7αH-germacra-1(10)*E*,4(15)-diene-2-acetoxy,5α,6β-diol; **l** 1α,10β-epoxy-7αH-germacr-4(15)ene-2β,5α,6β-triol; **m** hymenovin; **n** hymenoratin B; **o** hymenoratin B 2-O-β-D-(6'-O-acetyl)-glucopyranoside; **p** floribundin; **r** lemmonin A; **s** 2α-tigloyloxydugaldiolide; **t** neohymenoratin; **u** hymenoratin; **v** acetylhymenograndin.

Tab. 1 The colours of the spots of selected sesquiterpenoids isolated from Asteraceae family.

No.	Compound	Plant	Colour of the spot in anisaldehyde reagent
1	stizolin	*Stizolophus balsamita*	mauve
2	stizolicin		
3	cebellin M	*Centaurea bella*	
4	9α-hydroxyparthenolide	*Zoegea baldschuanica*	violet
5	salonitenolide	*Centaurea crocodylium*	red-brown
6	cnicin		
7	stenophyllolide	*Centaurea lusitanica*	black-blue
8	4β,5α-epoxy-7αH-germacr-1(10)*E*-ene-2β,6α-diol	*Santolina neapolitana*	dark-blue
9	2-acetoxy, 4β,5α-epoxy-7αH-germacr-1(10)*E*-ene-6β-ol		
10	7αH-germacra-1(10)*E*,4(15)-diene-2β,5α,6β-triol		mauve
11	7αH-germacra-1(10)*E*,4(15)-diene-2-acetoxy,5α,6β-diol		
12	1α,10β-epoxy-7αH-germacr-4(15)ene-2β,5α,6β-triol		
13	hymenoratin B	*Helenium hoopesii*	orange
14	hymenovin		
15	hymenoratin B 2-O-β-D-(6'-O-acetyl)-glucopyranoside		yellow
16	floribundin		lack of colour
17	lemmonin A		cherry-red
18	2α-tigloyloxydugaldiolide		
19	neohymenoratin		
20	hymenoratin		
21	acetylhymenograndin		gray

TLC analysis

Thin-layer chromatography was performed at room temperature on aluminium-backed silica gel plates DC Alufolien Kieselgel 60 (Merck art. 5553). 15-20 μg of each isolated compounds were applied per plate. Developed and dried chromatograms were sprayed by anisaldehyde reagent and heated at 100° C by 3 minutes. Spots of isolated compounds exhibited, mauve, violet, red-brown, black-blue, orange, yellow, cherry-red and gray colours when examined 3-15 minutes from the time of spraying (Tab. 1).

Results

Germacranolides

The characteristic colour of the spots after spraying with the anisaldehyde reagent of the separated compounds from the aerial parts of *Stizolophus balsamita* [5], *Centaurea bella* [3,6], *Zoegea baldschuanica* [4], *Centaurea crocodylium* and *Centaurea lusitanica* [6] can be ascribed to the presence or lack of several substituents at C8 (Fig. 2). Two germacranolides: stizolin (Fig. 1a) and stizolicin (Fig. 1b) of *Stizolophus balsamita* exhibit, mauve spots on chromatograms, apparently due to the very substituents. It is worth mentioning, that cebellin M (Fig. 1c) – the only one germacranolide among 25 guaianolides found in *Centaurea bella*, with OH group in C8 position, exhibits mauve spots as stizolin (Fig. 1a, Fig. 2a) on chromatograms. It can be inferred from the TLC results of 9α-hydroxyparthenolide (Fig. 1d, Fig. 2b), the germacranolide

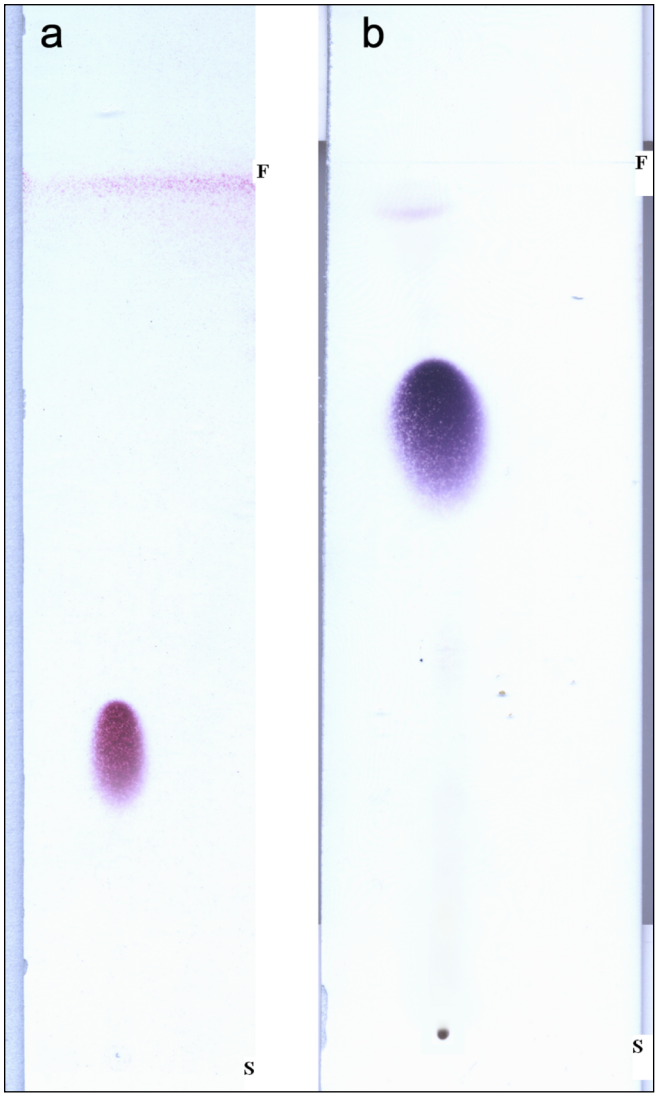

Fig. 2 Chromatogram of 8α-hydroxyparthenolide (stizolin). **a** Mobile phase: CH_2Cl_2— $CO(CH_3)_2$ 3:1 and 9α- hydroxyparthenolide. **b** Mobile phase: CH_2Cl_2— $CO(CH_3)_2$ 5:1. Adsorbent: silica gel; reagent: anisaldehyde. S – start; F – finish.

isolated from *Zoegea baldschuanica*, devoid of the substituent at C8, which makes them change the colour of the spots into violet (Fig. 2).

The sesquiterpene lactones in *Centaurea crocodylium* – salonitenolide (Fig. 1e) and its ester derivative – cnicin (Fig. 1f), have a different structure in comparison to germacranolides from *Stizolophus balsamita* (Fig. 1a,b). Both possess a characteristic methylhydroxy group at C4, a substituent at C8, and change their colour into red-brown on the chromatograms. Non 4,5-epoxy, with OH at C9, derivative of 9α-hydroxyparthenolide (Fig. 1d) – stenophyllolide (Fig. 1g), isolated from *Centaurea lusitanica* shows black-blue colour of spots on the chromatogram.

Biosynthesis of cnicin during the drying of *Centaurea crocodylium* herb

While an attempt was being made to obtain compounds of *C. crocodylium* by column chromatography [silica gel, CH_2Cl_2— $CO(CH_3)_2$ 8:1], from the extract from the fresh herb on large quantity (665 mg), salonitenolide (Fig. 1e) was isolated. Another compound, also in large quantity (845 mg), was obtained [silica gel, CH_2Cl_2— $CO(CH_3)_2$ 4:1] from the dry herb of the plant. To our astonishment, rather than salonitenolide (Fig. 1e), it turned out to be its ester derivative: cnicin (Fig. 1f). It can be stated, thus, that at the time of drying, the ester had been connected to the hydroxyl group at C8 of **e** on Fig. 1. It is also worth noting that with the methods used so far in this work, the attempts to isolate either cnicin (Fig. 1f) from the fresh herb, or salonitenolide (Fig. 1e) from the dried herb have been unsuccessful.

Germacranes

Two out of the five germacranes of *Santolina neapolitana* [7], with the 4,5 epoxide and a substituent at C2 (Fig. 1i) exhibit dark-blue colour of their spots on the chromatograms (Fig. 3a,c). The spots of compounds **j**, **k**, **l** on Fig. 1 have a different, namely mauve, colour (Fig. 3b,d,e). They lack of the 4,5-epoxide ring and are characterised by the presence of the OH group at C5 and the $=CH_2$ substituent at C4. The 1,10 epoxide of **l** on Fig. 1 has no influence on the colour of the spot.

Seco-pseudoguaianolides

Another type of sesquiterpene lactones occurs in the herb of *Helenium hoopesii* [8], with seco-pseudoguaianolides (Fig. 1m-p) as the dominant feature. Hymenovin – mixture of C3 and/or C4 diastereoisomers (Fig. 1m) and hymenoratin B (Fig. 1n) with pyran skeleton with at least one OH group at C2 or C3 and C4 exhibited orange colour on the chromatograms. Hymenoratin B 2-O-β-D-(6'-O-acetyl)-glucopyranoside (Fig. 1o), on the other hand, with a pyran ring and blocked hydroxyl

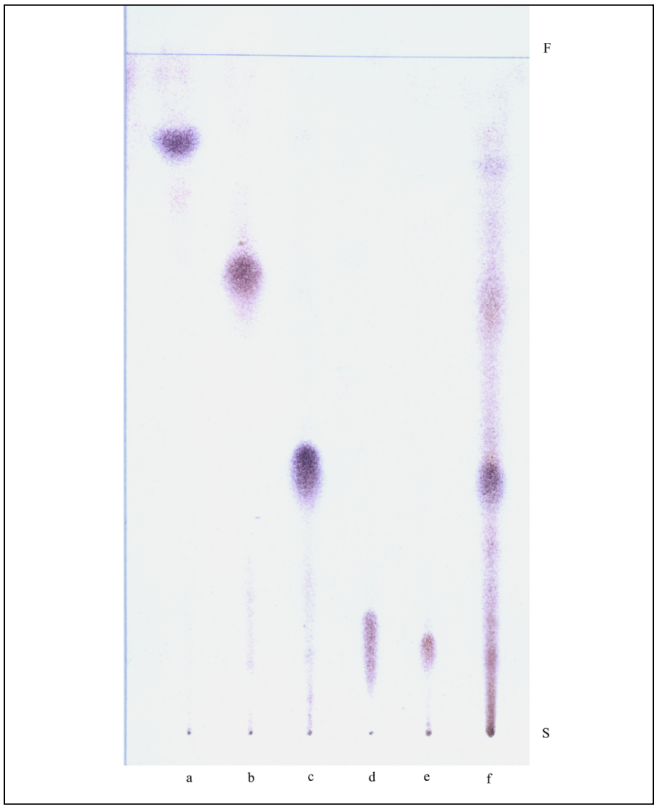

Fig. 3 TLC of germacranes of *Santolina neapolitana* herb. **a** 2-acetoxy,4β,5α-epoxy-7αH-germacr-1(10)*E*-ene-6β-ol; **b** 7αH-germacra-1(10)*E*,4(15)-diene-2-acetoxy,5α,6β-diol; **c** 4β,5α-epoxy-7αH-germacr-1(10)*E*-ene-2β,6β-diol; **d** 7αH- germacra-1(10)*E*,4(15)-diene-2β,5α,6β-triol; **e** 1α,10β-epoxy-7αH-germacr-4(15)ene-2β,5α,6β-triol. **f** The extract from the herb of *Santolina neapolitana*. Adsorbent: silica gel; mobile phase: $CHCl_3$— EtOAc 6:1; reagent: anisaldehyde. S – start; F – finish.

groups at C2 and C4, changes its colour to yellow on the chromatograms, while floribundin (Fig. 1p), without a substituent at these places, is invisible in the anisaldehyde reagent (but fast crystallizes as white, spectrally clear needles). This compound was presented in this paper for the first time as one of compounds isolated from *H. hoopesii*.

Guaianolides and pseudoguaianolides

The other compounds of this plant: guaianolides (Fig. 1r,s) and pseudoguaianolides (Fig. 1t,u) with a five-part ring exhibited cherry-red colour. The fading of the cherry-red colour can be observed in acetylhymenograndin (Fig. 1v). Three acetyl groups in this compound are responsible for the grey colour of the spot.

Conclusions

Sesquiterpenoids constitute an important group of natural compounds occurring in species of family Asteraceae. By means of the simple TLC method, it is possible to estimate several details of the structure within guaianolides, germacranolides, pseudoguaianolides, seco-pseudoguaianolides and germacranes.

The simple thin layer chromatography may surely rank among methods of comparative analysis of natural compounds. It is often easier to differentiate and classify species based on TLC than through the traditional botanical analysis. One might assume that with high probability. *Zoegea* species will biosynthesize "violet" germacranolides with substituent at C9, and that *Stizolophus* species will biosynthesize "mauve" germacranolides with substituents at C8, and some *Centaurea* species will biosynthesize "brown-red" ones, with the characteristic CH_2OH group at C4.

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