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Determination of taxa of the *Achillea millefolium* group and *Achillea crithmifolia* by morphological and phytochemical methods I. Characterisation of Central European taxa[1]

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Abstract

A survey of morphological and phytochemical data characteristic for several taxa of the *Achillea millefolium* group (*A. aspleniifolia* VENT., *A. roseoalba* EHREND., *A. collina* J. BECKER ex REICHENB., *A. ceretanica* SENNEN, *A. setacea* W. ET K., *A. pratensis* SAUKEL & LÄNGER, *A. styriaca* SAUKEL ined., *A. pannonica* SCHEELE, *A. distans* W. ET K., *A. millefolium* s.l., *A. millefolium* ssp. *sudetica* OPIZ) and *A. crithmifolia* W. ET K. is presented. For each taxon a short morphological description and a guide for microscopic analysis is given as well as its sesquiterpene composition including the TLC characteristics. Based on GLC analyses of 1523 single plants collected in Central Europe the sums of sabinene + β-pinene + β-caryophyllene (SUM 1), α-pinene + 1,8-cineole (SUM 2), camphene + camphor + borneol (SUM 3), camphene + camphor (SUM 4) and 1,8-cineole + borneol (SUM 5) were found to be highly significant for distinct taxa or groups of taxa.

Keywords

Achillea millefolium, sesquiterpenoids, GLC analysis, monoterpenes, morphometry, ploidy, crude drug analysis

[1] Up to now 2600 single plants from Central and Southeast Europe were investigated. In the present paper the results of 1523 Central European plants are discussed.

Introduction

Yarrow is a widespread plant used in folk medicine due to its various effects. The indications include gastric and intestinal disorders, inflammation of skin and mucosa as well as hemorrhages (Wichtl, 1997). The sesquiterpenes were shown to contribute to the antiphlogistic effects (Della Loggia *et al.*, 1992; Kastner *et al.*, 1993; Sosa *et al.*, 2001). The fact that the *Achillea millefolium* group consists of several different taxa (Ehrendorfer, 1953; Saukel *et al.*, 1992, 1992a, 1992b, 2002) and that in folk medicine herbal teas are prepared without regard to the species led to intensive morphological and phytochemical investigations. The latter were focused on sesquiterpenes and essential oil, both showing chemotaxonomic relevance (Kastner *et al.*, 1992; Kubelka *et al.*, 1999). The present paper gives an overview of the different taxa including brief morphological descriptions, pictures of typical leaflets and rayflorets, a summary of the sesquiterpenoids isolated up to now and the essential oil composition. Linear combination of the essential oil compounds is introduced as a new special feature which is highly significant for distinct taxa or groups of taxa.

Although *A. crithmifolia* does not belong to the *A. millefolium* group it is taken into consideration in the present paper. It was detected frequently in drug material available in Austrian pharmacies (Rehberger, 1996) and contains rupicolines which might trigger allergic reactions due to their exocyclic methylene group. The large amount of the obtained data is based on the analyses of more than 2600 single plants including plants from East Europe. This paper deals with those which were collected in Central Europe and which are of pharmaceutical relevance (1523 single plants). The combination of the presented morphological and phytochemical characteristics also allows the determination of unidentified drug material.

Experimental

Plant material

The numbers of morphologically and chemically examined specimens of each *Achillea* species are: *A. asplenifolia* – N = 233; *A. roseoalba* - N = 63; *A. collina* - N

= 191; *A. ceretanica* – N = 30; *A. crithmifolia* - N = 19; *A. setacea* - N = 142; *A. pratensis* - N = 105; *A. styriaca* - N = 74; *A. pannonica* - N = 174; *A. distans* - N = 54; *A. millefolium* s.l. - N = 320; *A. millefolium* ssp. *sudetica* - N = 118.

Morphometry

The habitus was described by measuring defined marks (e.g. plant-length, stem-diameter, length of an upper leaf etc.). The rayflorets and the leaflets are of high systematic relevance within the *Achillea millefolium* group (Saukel and Länger 1992, 1992a). Their outlines were painted from microscopic preparations, digitalised and measured by self-designed programs (Rehberger, 1996; Nejati, 2002; Rauchensteiner, 2002; Saukel, 2002a ined.).

Flowcytometry

Ploidy level was evaluated by cutting some leaflets of an upper stem-leaf. For higher ploidy levels the experiment was employed together with a diploid standard (cloned *A. ceretanica* 2x) and in case of diploid samples together with a tetraploid standard (cloned *A. pratensis* 4x) in a citric acid-buffer medium. After filtration, 4,6-diamidino-2-phenyl-indole together with sulphorhodamine were added and 5 minutes later the sample was measured (for details see Wlach 2002). We used a Partec–CCA 1 (Cell Counter Analyser) equipped with a Hg-lamp as a source for UV-radiation and a fluorescence-detector including a photomultiplier.

Sample preparation for GLC

100 mg flower heads were extracted with 1 ml dichloromethane for 10 minutes in an ultrasonic bath. 1.5-2.0 µl of this solution were injected, the rest was evaporated under nitrogen, redissolved in 200 µl dichloromethane and used for TLC.

GLC analysis – parameters and identification of the substances

The analyses were performed on a Perkin Elmer AutoSystem XL Gas Chromatograph. Column: Hewlett Packard HP-5 (crosslinked 5% PhMe Silicone), 50 m

x 0.32 mm x 0.52 µm film thickness. Carrier gas: nitrogen 5.0 (2 ml/min), split ratio 1:10. Detection: FID (hydrogen 5.0, synthetic air 5.0). Temperature: injector 270°C, detector 280°C, oven program: 60-270°C, rate: 3°C/min. Identification of the substances was performed by retention indices and co-injection of reference substances. Kovats-indices: α-pinene 942, camphene 958, sabinene 980, ß-pinene 984, p-cymene 1030, 1,8-cineole 1039, camphor 1157, borneol 1174, bornylacetate 1302, ß-caryophyllene 1437.

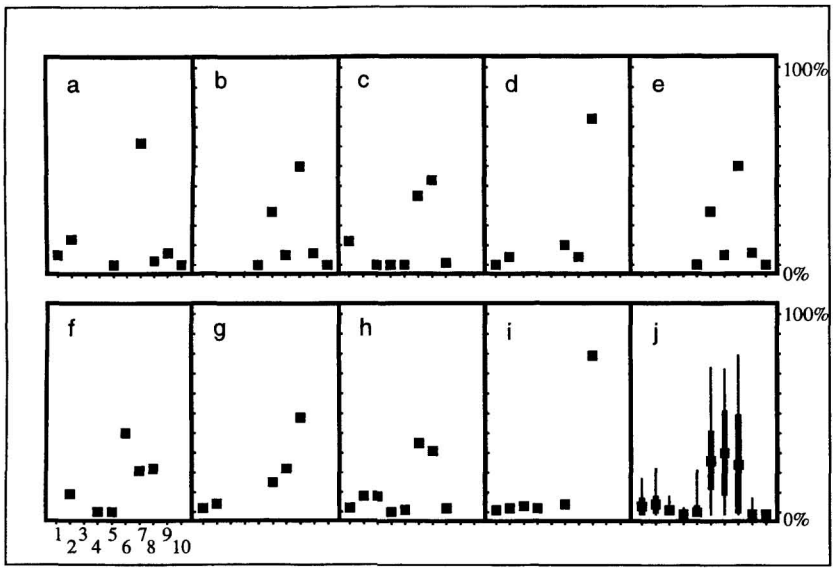


Figure 1. Relative amounts of the terpenes 1-10 (1- α-pinene, 2- camphene, 3- sabinene, 4- ß-pinene, 5- p-cymene, 6- 1,8-cineole, 7- camphor, 8- borneol, 9- bornylacetate, 10- ß-caryophyllene) in nine single plants of *A. setacea* (**a - i**) and the sum of all 142 samples of *A. setacea* (**j**, black square – mean, black rectangle – standard deviation, line – range).

Essential oil – sum parameters and calculation

Computer guided classification and comparison of the different taxa based on the essential oil required the determination of distinct parameters. According to oc-

currence and reproducibility nine monoterpenes and ß-caryophyllene were chosen and used for all computations, as their peak areas showed linear correspondence with concentrations (Rauchensteiner *et al.*, 2002a ined.). The peak areas of the chosen substances were summarised, set 100% and recalculated, which permits a comparison of the single plants independent of the injected amount. However, within all the taxa of the genus *Achillea* a great variability of the data is observed. In none of the investigated species one of the compounds shows stable values. E. g. in *A. setacea* the values of 1,8-cineol, camphor and borneol vary between 0 and 80% (fig. 1, a – i, j). The analyses of the correlation coefficients show for *A. setacea* –0,82 (camphor against borneol) in the case of *A. collina* –0,84 (sabinene against ß-pinene) and for *A. pannonica* –0,74 (α-pinene against 1,8-cineol). An extensive examination of the data material led to the conclusion that especially the sums of sabinene + β-pinene + β-caryophyllene (**SUM1**), α-pinene + 1,8-cineole (**SUM2**) and camphene + camphor + borneol (**SUM3**) are very characteristic for distinct taxa or groups of taxa: Fig. 2 shows a scatterplot for the sums of the proazulene containing species (**proaz**), *A. setacea* (**set**), *A. pannonica* (**pan**) and *A. styriaca* (**styr**), respectively. It is clearly visible that the oils of *A. pannonica* and the proazulene group are fully described by **SUM 1** and **SUM 2**, whereas *A. setacea* is characterised by small values of **SUM 1** and small to high values of **SUM 2**. Additional linear combinations were found for camphene + camphor (**SUM 4**) and 1,8-cineole + borneol (**SUM 5**) shown in fig. 3 with the same species as in fig. 2. *A. setacea* is represented with high significance by SUM 4 and SUM 5. *A. styriaca* shows some similarities but the different position is due to additional compounds in the oil.

The sum parameters 1, 2, 3 were therefore plotted in ternary graphs (tab. 2a - l). Within these three coordinates each single plant is determined by one distinct data point revealing characteristic values for the respective taxon.

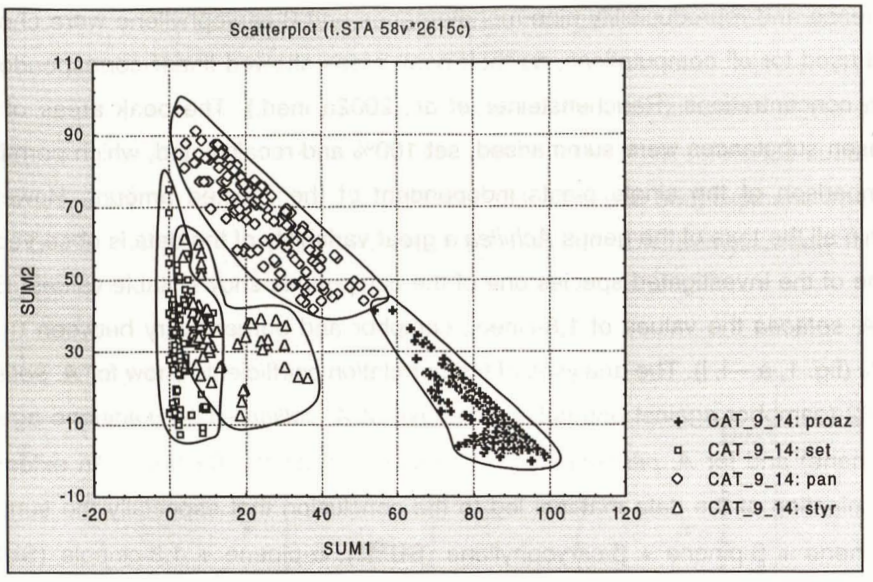


Figure 2. Comparison of proazulene containing taxa (**proaz**), *A. setacea* (**set**), *A. pannonica* (**pan**) and *A. styriaca* (**styr**) based on **SUM 1** and **SUM 2** (see text).

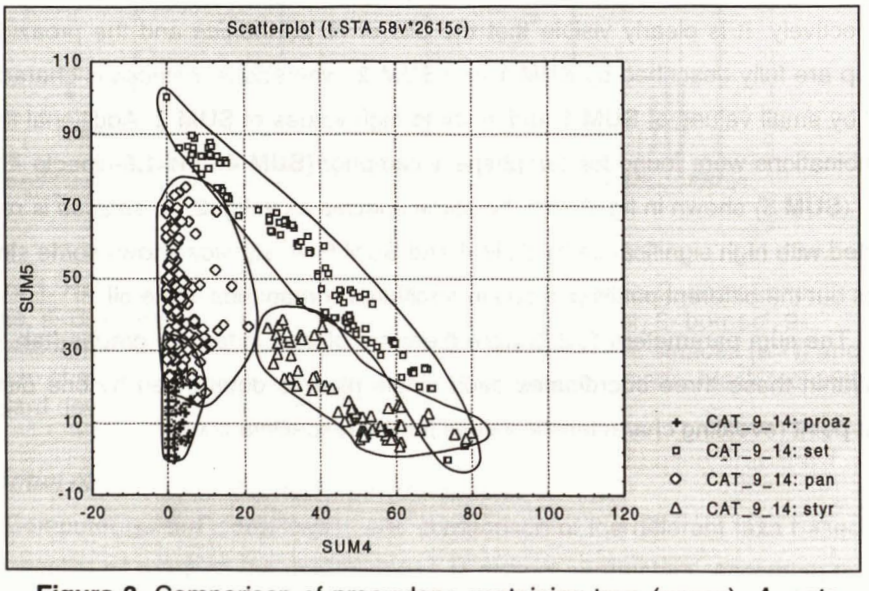


Figure 3. Comparison of proazulene containing taxa (**proaz**), *A. setacea* (**set**), *A. pannonica* (**pan**) and *A. styriaca* (**styr**) based on **SUM 4** and **SUM 5** (see text).

TLC

Silica gel 60 Merck plates (0.25 mm) were developed with dichloromethane-acetone (9+1) as mobile phase (room temperature). As reference a dichloromethane extract of camomile was prepared (reference substance: matricin). Developed plates were examined under $UV_{255\ nm}$. Additionally modified acetic acid-phosphoric acid reagent (Stahl, 1967) served as selective and very sensitive detection reagent for the proazulenes giving blue-green coloured spots after heating at 140°C. The treatment of the sprayed plate with water steam causes a change of the colour into bright blue as well as a remarkably intensification of the stains. Subsequently the same TLC was sprayed with anisaldehyde sulphuric acid reagent (Dequeker, 1964) and heated to visualise the non-proazulenes.

Quick-Testing for proazulenes and other sesquiterpene lactones

One capitulum was placed on a slide and heated over a flame after addition of a small amount of CP-reagent (60 % chloralhydrate - 85 % phosphoric acid (2+1); Saukel, 1993). This procedure makes it easier to prepare the rayflorets. The reaction of the glands was observed in the microscope – a blue or black colour indicated the presence of proazulenes whereas any other or missing colour indicated a different composition within the sesquiterpene lactones (tab. 2a - l). An important requirement is to use only dried plant material – fresh material gives in most cases a wrong positive reaction, even though proazulenes lack.

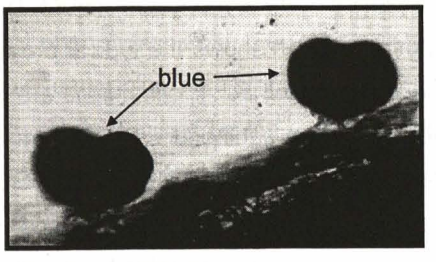


Figure 4. Blue colour of the glands indicating proazulenes.

Results and Discussion

Twelve Central European taxa were investigated with regard to the morphology, the essential oil composition and the sesquiterpene pattern. For the morphological characterisation the morphology of the leaflets and rayflorets turned out to be most suitable (Saukel *et al.*, 1992, 1992a, 1992b,1994, 2002).

The essential oils of 1523 single plants collected in Central Europe were examined by GLC. Each tested individual showed a stable composition (Kastner *et al.*, 1992) whereas plants of one species differed in the content of their main compounds (fig. 1). By summing up two or three out of ten of these compounds we got stable values, the sums (see experimental), which allowed the assignment of the samples to distinct species or to aggregates of species. Extensive investigations of the whole data material revealed significant negative correlations of the terpenes used for the sum parameters. In the diagrams the single datapoints are located within a triangle, each of the three corners represents 100% of one of the five sum parameters. As a result the data of a large number of single plants can be shown in one diagram allowing a comparison between the different taxa. The plots with the sum parameters 1, 2 and 3 (tab. 2a – l) showed the same phenomenons and tendencies as those with the sum parameters 1, 4 and 5. Remarkably seems that no datapoint was located on the axis between sum parameter 1 and 3.

As result the proazulene containing species *A. aspleniifolia*, *A. roseoalba*, *A. collina* and *A. ceretanica* clearly show homogeneity of the essential oil with a high content of sabinene + ß-pinene + ß-caryophyllene (SUM 1). This is confirmed by the sesquiterpene composition which shows the typical proazulene pattern (tab. 3, fig. 5). In contrast, *A. setacea* as well as *A. crithmifolia* are located along the axis between SUM 2 and SUM 3 lacking sabinene + ß-pinene + ß-caryophyllene (SUM 1). *A. crithmifolia* is characterised by a higher content of SUM 2 than *A. setacea* and has four types of sesquiterpene skeletons. Their shape of leaflets differ clearly from each other (tab. 2e, f) whereas both species have very similar rayflorets and contain rupicolines A and B (tab. 3). These are polar guaianolides with an exocyclic methylene group giving blue-green spots on TLC after spraying with acetic acid-

phosphoric acid reagent (Zitterl-Eglseer *et al.*, 1991) and pale green coloured glands with CP-reagent. The essential oil characteristics of *A. styriaca* resemble to those of *A. setacea* but the shape of the leaflets is different. The surprising number of sesquiterpene skeletons (eudesmane, germacrane and longipinane) could partly be explained by a hybridisation process with *A. pratensis*.

The remaining taxa *A. pratensis*, *A. pannonica*, *A. distans*, *A. millefolium* ssp. *sudetica* and *A. millefolium* s.l. show high variability and overlappings so that based on the essential oil only, the assignment to a certain taxon will fail. Only *A. pratensis* is characterised by one sesquiterpene type (eudesmanolides). The hexaploid *A. millefolium* (incl. ssp. *sudetica*) shows three types (guaianolides, germacranes and eudesmanolides), *A. pannonica* four types (guaianolides, germacranes, longipinanes and farnesane) of sesquiterpenes.

The scatterplot of *A. millefolium* s.l. (tab. 2k) includes a large number of single plants (320) and gives an impression of the heterogeneity within that group, as the individuals are spread almost over the whole area of the triangle. 89 individuals from Northwest Europe show a high value of SUM 1 and therefore resemble to proazulene containing species. For differentiation a check of the sesquiterpene pattern by TLC and/or morphological investigations (the value of the diameter of pollengrains and the dimension of the rayflorets are very high) have to be performed. In accordance to its ploidy level (6x), *A. millefolium* s.l. can be described as a polyphyletic species with many phenotypes depending on the geographical origin. From the more or less distinct *A. millefolium* ssp. *sudetica* in mountainous and alpine habitats also several phenotypes exist (Saukel in: Adler *et al.* 1994). As a consequence, *A. styriaca*, *A. pannonica*, *A. distans*, *A. millefolium* ssp. *sudetica* and *A. millefolium* s.l. can only be determined by the combination of morphology, sesquiterpene pattern and essential oil composition.

Conclusion

The essential oil represents a helpful tool to distinguish between proazulene and non-proazulene containing species of taxa of the *A. millefolium* group and *A.*

crithmifolia. Sum parameters of the essential oil components correspond to morphological features in most cases. A discriminant analysis of the di-, tetra- and octoploid species (all proazulene containing taxa were concentrated in one group) was performed with all five sum parameters and the contents of p-cymene and bornylacetate. The analysis revealed a correct assignment for the proazulene containing group with 99,7%, *A. pannonica* 97,1%, *A. setacea* 94,3%, *A. styriaca* 93,2% and *A. pratensis* 87,8%. Consequently the presented results can also be combined to identify unknown drug material. A key for determination is given in tab. 1. Up to now this key is valid only for uncomminuted drugs, as leaflets and flowers from one plant are required to obtain correct results. The identification of cut drug requires a modification of the key which is in progress.

1	glands deep blue or magenta or black, SUM 1 >70		2
1´	glands pink or auburn, SUM 2 <40 or SUM 2 > 60		3
1´´	glands pale green		4
1´´´´	glands uncoloured		5
2	glands deep blue or magenta or black, length of corolla tube divided by length of ligule <0,9	***A. aspleniifolia, A. roseoalba A. ceretanica***	
2´	glands magenta or black, length of corolla tube divided by length of ligule >1,0	***A. collina***	
3	SUM 2 >60, diameter of pollengrains > 36,5 µm	***A. pannonica***	
3´	SUM 2 <40, diameter of pollengrains < 35,0 µm length of leaflets divided by width of leaflets >1,9, with longipinenes	***A. styriaca***	
4	all glands greenish, width of leaflets rachis < 0,4 mm, length of corolla tube divided by length of ligule > 1,0	***A. setacea***	
4´	all glands greenish, width of leaflets rachis > 0,4 mm, involucrum onion shaped, length of corolla tube divided by length of ligule > 1,0	***A. crithmifolia***	
4´´	only some glands greenish, rayflorets large (length > 2,3mm, width > 2,5 mm), diameter of pollengrains > 36 µm	***A. millefolium*** (incl. ssp. ***sudetica)***	
5	with eudesmanolides	***A. pratensis***	
5´	without eudesmanolides, rayflorets large (length > 2,3mm, width > 2,5 mm), diameter of pollengrains > 36 µm		6
6	length of leaflets divided by width of leaflets > 2,0	***A. distans***	
6´	length of leaflets divided by width of leaflets < 1,8	***A. millefolium*** **(incl. ssp. *sudetica*)**	

Table 1. Determination key for uncomminuted drugs of taxa of the *A. millefolium* group and *A. crithmifolia*.

A. aspleniifolia VENT., N = 233

Morphological and Cytological Features

Ploidy level: di- (or sometimes tetraploid)

Morphology: usually tenderly, curved stem, not branched, with long internodes in the lower part; leaves of the lower part distinctly petiolate, broad rachis; leaflets nearly planar, with white collenchymatic margin and stinging tips; pink or white rayflorets with ligule as long as broad.

Width/length of leaflets: 4,4/3,4 mm

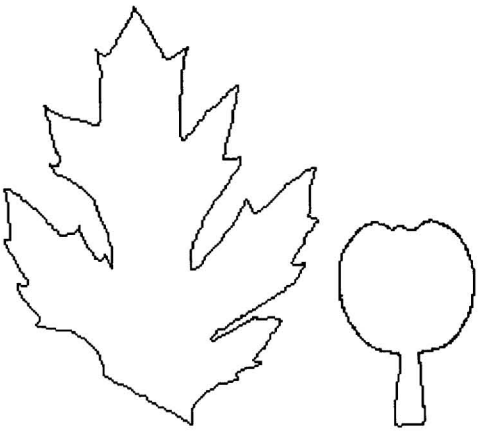
Width of the leaflets rachis: 1,0 mm

Width/length of involucrum: 2,6/3,7 mm

Width/length of ligule: 2,1/2,0 mm

Corolla tube length divided by ligule length: 0,84

Diameter of pollengrains (with spines): 32,4 µm

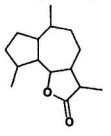


Chemical Features

GLC-characteristics: Dominant ß-pinene with high amounts of sabinene and ß-caryophyllene

Colour of glands: deep blue (rarely dark grey to black)

Sesquiterpene skeleton:



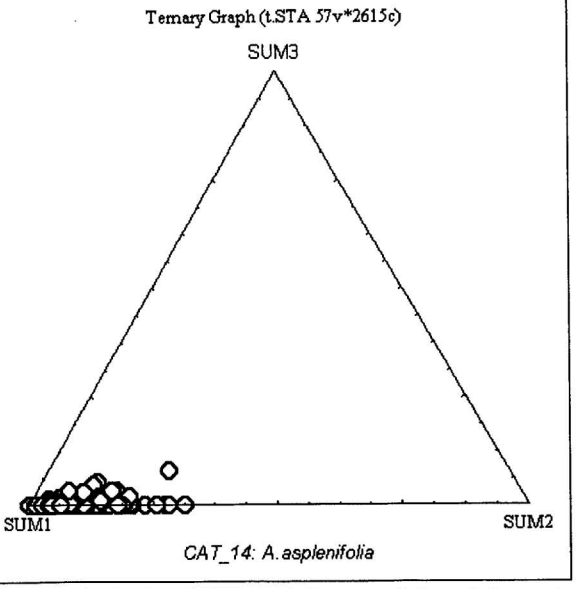


Table 2a. Short morphological, cytological and chemical characteristics of *A. aspleniifolia*.

A. roseoalba EHREND., N = 63

Morphological and Cytological Features

Ploidy level: diploid

Morphology: usually not tall, tenderly, not branched, with small inflorescense, low number of nodes; leaves fine, leaflets with extended parts; pink rayflorets with relatively long ligule.

Width/length of leaflets: 5,4/3,5 mm

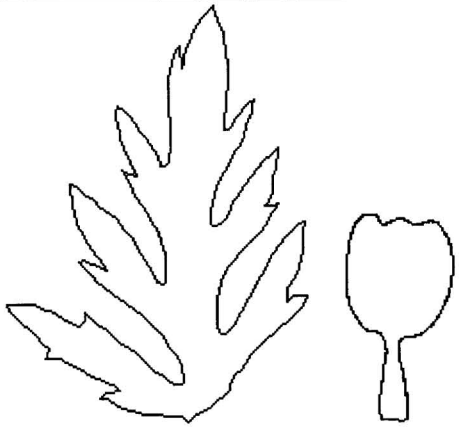
Width of the leaflets rachis: 0,87 mm

Width/length of involucrum: 2,3/3,6 mm

Width/length of ligule: 2,0/2,0 mm

Corolla tube length divided by ligule length: 0,80

Diameter of pollengrains (with spines): 30,5 µm

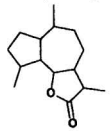


Chemical Features

GLC-characteristics: sabinene dominant with high amounts of ß-pinene and ß-caryophyllene

Colour of glands: dark grey to grey-magenta

Sesquiterpene skeleton:



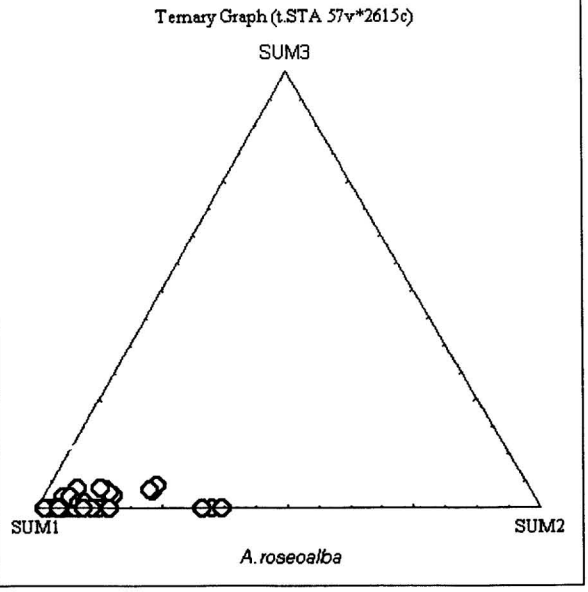


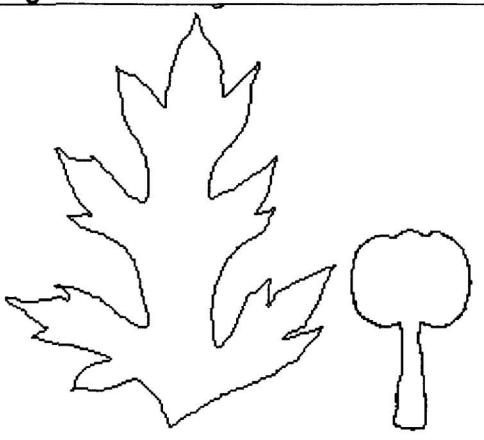
Table 2b. Short morphological, cytological and chemical characteristics of *A. roseoalba*.

A. collina J. BECKER EX REICHENB., N = 191

Morphological and Cytological Features

Ploidy level: tetraploid
Morphology: several morphotypes; plants often grayish –green and hairy; leaves always lanceolate (on dry places small), with high number of leaflets; white rayflorets with broad ligule, corolla tube as long or longer than ligule.

Width/length of leaflets: 3,1/4,3 mm
Width of the leaflets rachis: 0,65 mm
Width/length of involucrum: 2,7/3,7 mm
Width/length of ligule: 2,1/1,8 mm
Corolla tube length divided by ligule length: >1,0
Diameter of pollengrains (with spines): 33,1 µm

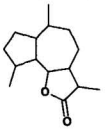


Chemical Features

GLC-characteristics: high amounts of sabinene or ß-pinene with smaller amounts of 1,8-cineole and ß-caryophyllene

Colour of glands: dark grey to grey-magenta to black

Sesquiterpene skeleton:



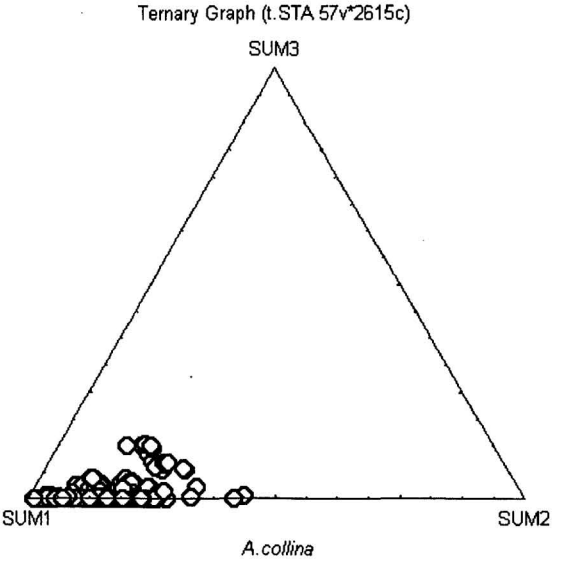


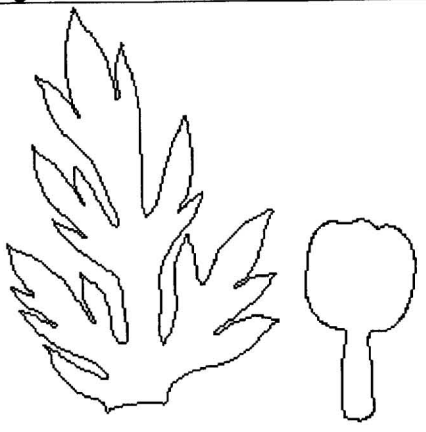
Table 2c. Short morphological, cytological and chemical characteristics of *A. collina*.

A. ceretanica SENNEN, N = 30

Morphological and Cytological Features

Ploidy level: di- and tetraploid
Morphology: plants often tenderly. grayish –green and hairy, with linear leaves, inflorescense small; leaflets small; white or pink rayflorets

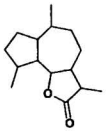
Width/length of leaflets: 2,4/3,1 mm
Width of the leaflets rachis: 0,6 mm
Width/length of involucrum: 2,7/3,8 mm
Width/length of ligule: 2,0/2,0 mm
Corolla tube length divided by ligule length: 0,74
Diameter of pollengrains (with spines): 33,6 µm



Chemical Features

GLC-characteristics: sabinene or and ß-pinene dominant

Colour of glands: dark grey to grey-magenta
Sesquiterpene skeleton:



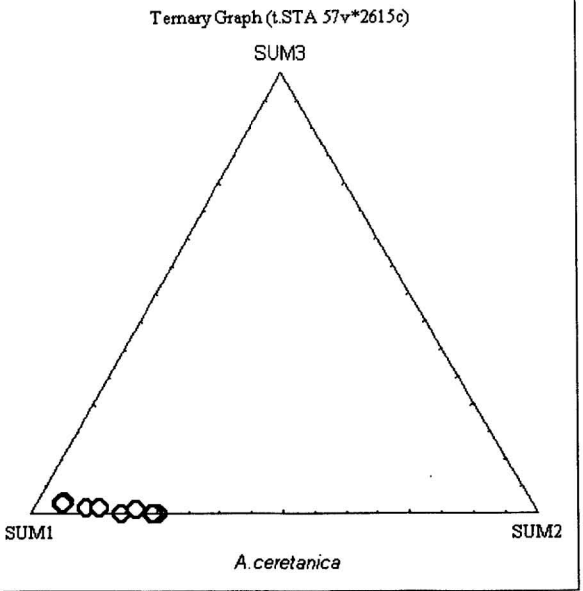


Table 2d. Short morphological, cytological and chemical characteristics of *A. ceretanica*.

A. setacea W. ET K., N = 142

Morphological and Cytological Features

Ploidy level: diploid

Morphology: plants small, grayish –green, densly hairy, with high number of nodes; leaves with high number of leaflets; parts of leaflets very fine divided; yellowish-white rayflorets, small with corolla tube longer than ligule

Width/length of leaflets: 2,1/2,4 mm

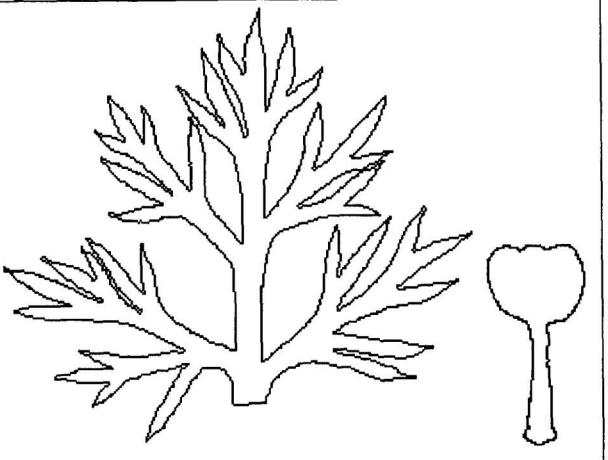
Width of the leaflets rachis: 0,33 mm

Width/length of involucrum: 2,5/3,8 mm

Width/length of ligule: 1,8/1,5 mm

Corolla tube length divided by ligule length: 1,35

Diameter of pollengrains (with spines): 29,0 µm

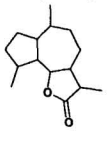


Chemical Features

GLC-characteristics: high amounts of 1,8-cineole or/and camphor or/and borneol

Colour of glands: light green

Sesquiterpene skeleton:



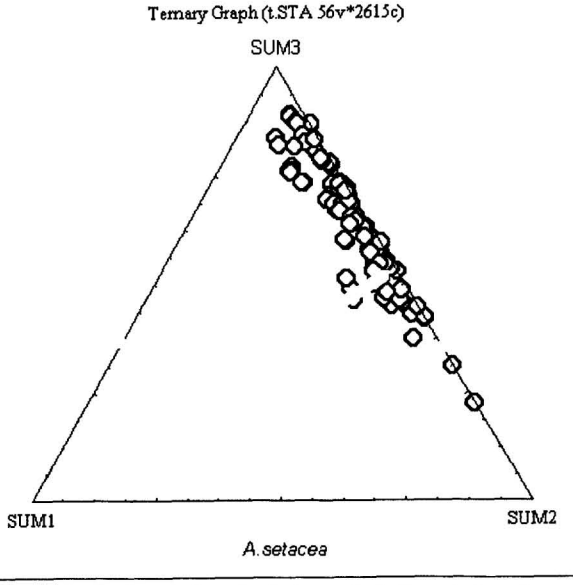


Table 2e. Short morphological, cytological and chemical characteristics of *A. setacea*.

A. crithmifolia W. ET K., N = 19

Morphological and Cytological Features

Ploidy level: di- and tetraploid

Morphology: grayish -green colour, no stolons, high number of nodes, more or less hairy; leaves oval; leaflets with broad upper segments; sulfuric-yellow rayflorets with characteristic and very thin corolla tube, the tube is longer than ligule

Width/length of leaflets: 3,9/6,4 mm

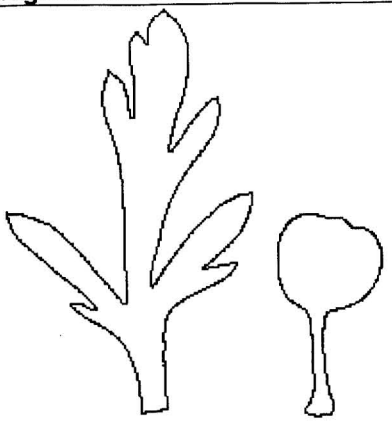
Width of the leaflets rachis: 0,89 mm

Width/length of involucrum: 2,0/3,3 mm

Width/length of ligule: 2,0/1,8 mm

Corolla tube length divided by ligule length: 1,1

Diameter of pollengrains (with spines): 28,4/32,5 µm

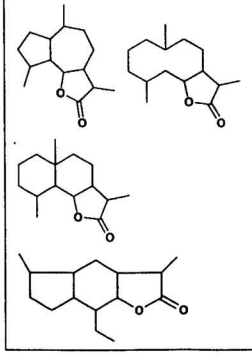


Chemical Features

GLC-characteristics: heterogenous, often high amounts of 1,8-cineole camphene and camphor

Colour of glands: light green

Sesquiterpene skeletons:



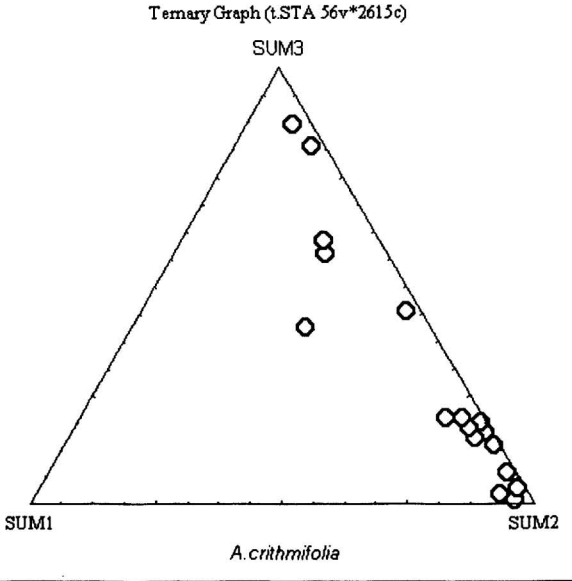


Table 2f. Short morphological, cytological and chemical characteristics of *A. crithmifolia*.

A. pratensis SAUKEL UND LÄNGER, N = 105

Morphological and Cytological Features

Ploidy level: tetraploid

Morphology: low number of nodes, very broad and big leaves; leaves with low number of leaflets; leaflets planar with a high number of tips; white or pink rayflorets.

Width/length of leaflets: 5,7/9,0 mm

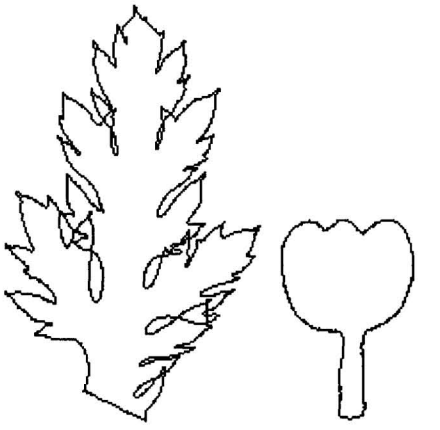
Width of the leaflets rachis: 1,1 mm

Width/length of involucrum: 3,2/4,1 mm

Width/length of ligule: 2,6/2,3 mm

Corolla tube length divided by ligule length: 0,76

Diameter of pollengrains (with spines): 34,3 µm



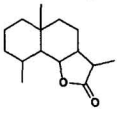
Chemical Features

GLC-characteristics:

heterogenous; often sabinen or/and ß-pinen or/and 1,8-cineole.

Colour of glands: *uncoloured*

Sesquiterpene skeleton:



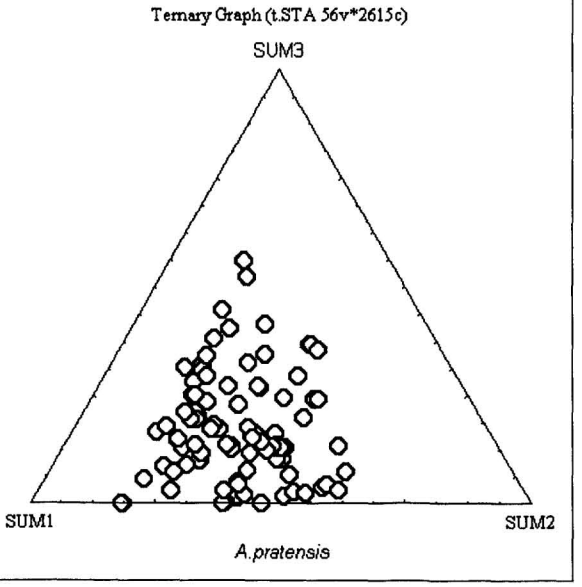


Table 2g. Short morphological, cytological and chemical characteristics of *A. pratensis*.

A. styriaca INED., N = 74

Morphological and Cytological Features

Ploidy level: tetraploid

Morphology: usually tall, not branched, hairy, compact inflorescense; leaves lanceolate, grayish-green, leaflets long with very high number of tips; white or pink rayflorets.

Width/length of leaflets: 6,1/11,6 mm

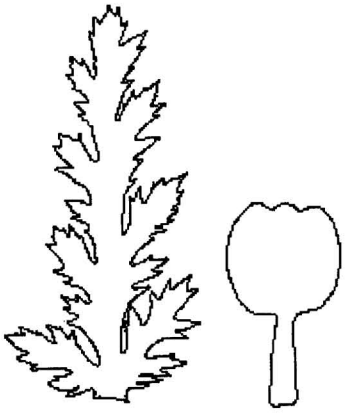
Width of the leaflets rachis: 1,28 mm

Width/length of involucrum: 2,4/4,0 mm

Width/length of ligule: 2,2/2,1 mm

Corolla tube length divided by ligule length: 0,77

Diameter of pollengrains (with spines): 32,5 µm

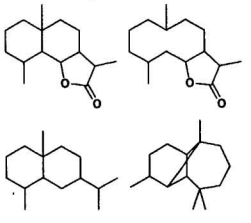


Chemical Features

GLC-characteristics: camphor dominant with high amounts of α-pinene or/and 1,8-cineole

Colour of glands: pink to auburn.

Sesquiterpene skeletons:



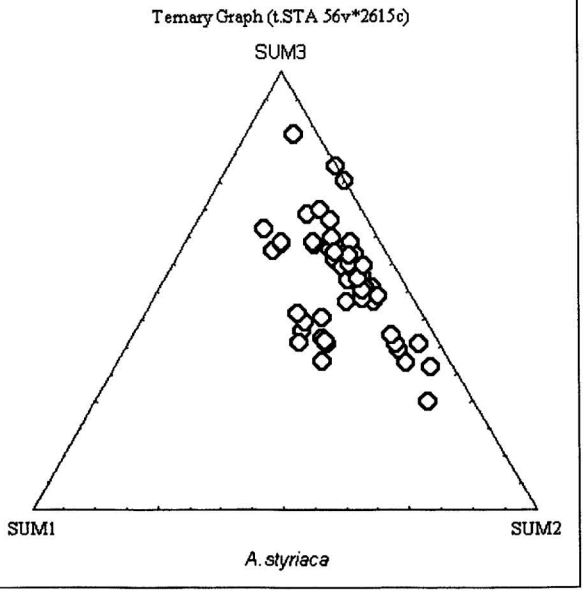


Table 2h. Short morphological, cytological and chemical characteristics of *A. styriaca*.

A. distans W. ET K., N = 54

Morphological and Cytological Features

Ploidy level: hexaploid

Morphology: low number of nodes; compact, large inflorescense; broad leaves with coarse, planar leaflets with high number of nodes; white or pink rayflorets, rayflorets big, with broad ligule.

Width/length of leaflets: 3,5/7,5 mm

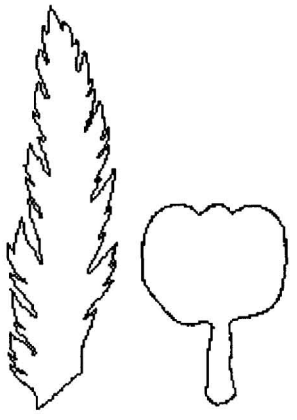
Width of the leaflets rachis: 1,4 mm

Width/length of involucrum: 3,5/4,5 mm

Width/length of ligule: 3,1/2,9 mm

Corolla tube length divided by ligule length: 0,71

Diameter of pollengrains (with spines): 37,1 µm



Chemical Features

GLC-characteristics:

high amounts of ß-pinene or/and 1,8-cineole and camphor

Colour of glands: uncoloured or light green.

Sesquiterpene skeleton: not yet investigated

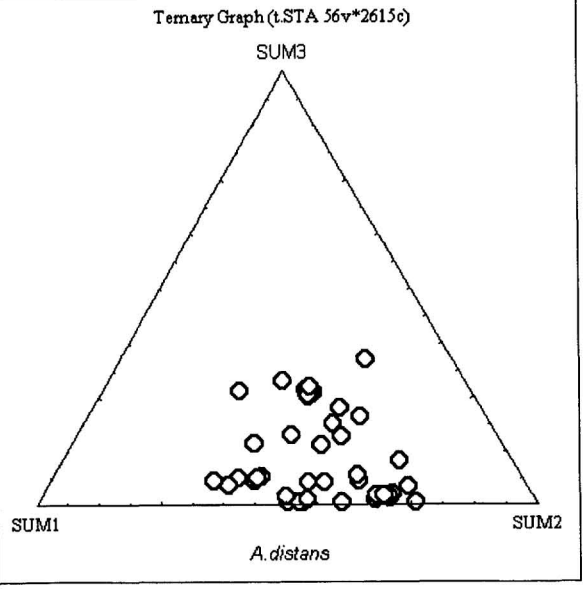


Table 2i. Short morphological, cytological and chemical characteristics of *A. distans*.

A. pannonica SCHEELE, N = 174

Morphological and Cytological Features

Ploidy level: octoploid (rarely hexaploid)

Morphology: grayish -green colour, rigid stem, very hairy; compact, large inflorescense and involucre; leaflets coarse, planar; white rayflorets with broad ligule and long corrolla tubes.

Width/length of leaflets: 3,1/4,2 mm

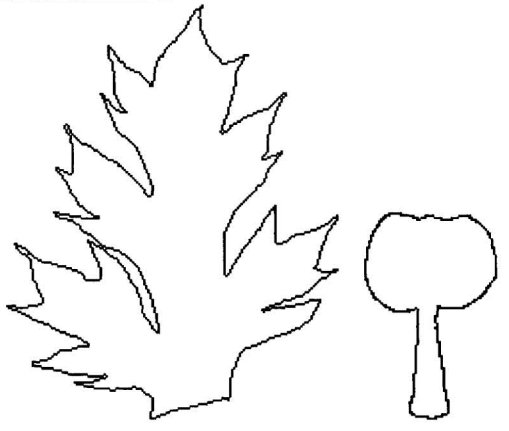
Width of the leaflets rachis: 0,82 mm

Width/length of involucrum: 3,2/4,5 mm

Width/length of ligule: 2,4/1,9 mm

Corolla tube length divided by ligule length: 0,96

Diameter of pollengrains (with spines): 37,5 µm

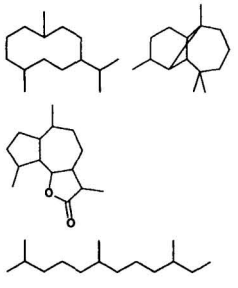


Chemical Features

GLC-characteristics: high amounts of 1,8-cineole or/and α-pinene, smaller amounts of sabinene or/and ß-pinene.

Colour of glands: uncoloured or pink.

Sesquiterpene skeletons:



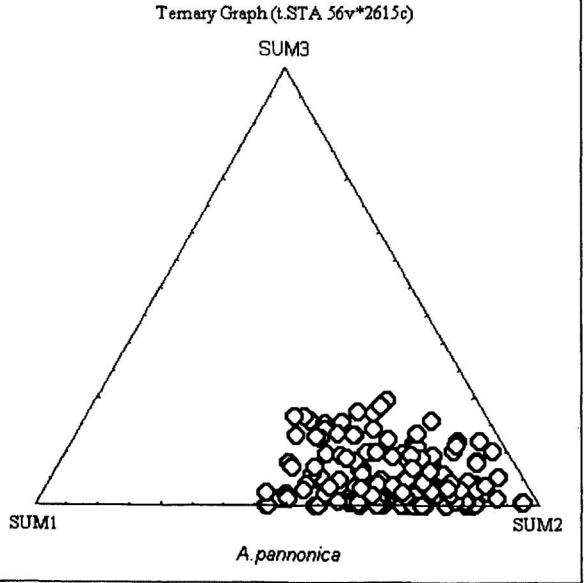


Table 2j. Short morphological, cytological and chemical characteristics of *A. pannonica*.

A. millefolium s.l., N = 320

Morphological and Cytological Features

Ploidy level: hexaploid

Morphology: heterogenous but stem allways strong; leafes lanceolate or broadly oval; leaflets medium to big; large white or pink rayflorets, corolla tube shorter than ligule

Width/length of leaflets: 3,1/4,6 mm

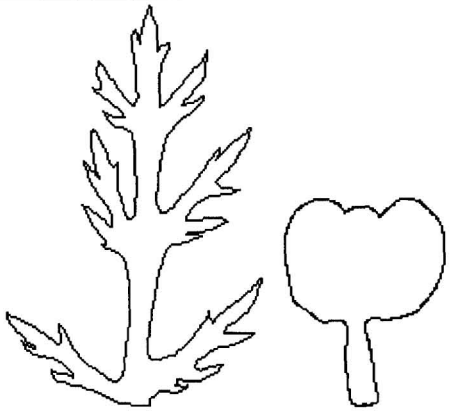
Width of the leaflets rachis: 0,7 mm

Width/length of involucrum: 3,7/4,6 mm

Width/length of ligule: 2,7/2,5 mm

Corolla tube length divided by ligule length: 0,7

Diameter of pollengrains (with spines): 36,0 µm

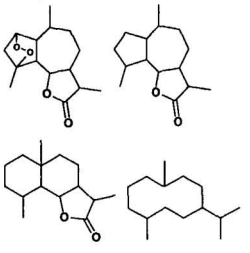


Chemical Features

GLC-characteristics: heterogenous; several chemotypes of different origin (see text)

Colour of glands: uncoloured or light green.

Sesquiterpene skeletons:



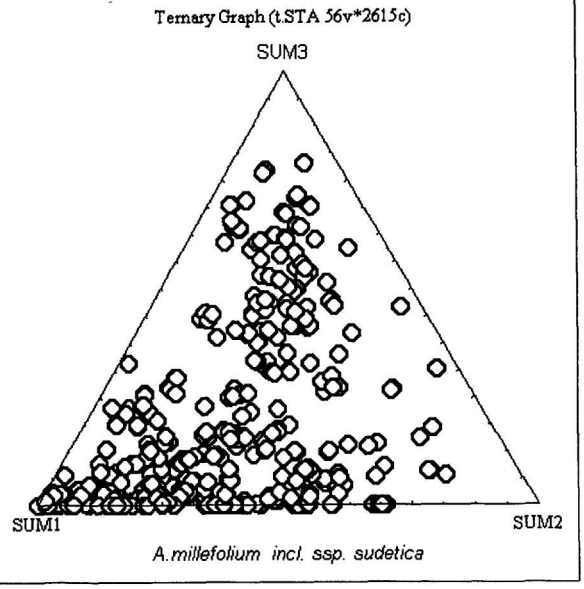


Table 2k. Short morphological, cytological and chemical characteristics of *A. millefolium* s.l.

A. millefolium ssp. *sudetica* OPIZ, N = 118

Morphological and Cytological Features

Ploidy level: hexaploid

Morphology: often short, with a rigid stem, broad and long basal leaves; leaflets long and broad, planar; rayflorets large, often pink, with large ligule and a short corolla tube. At last two morphotypes.

Width/length of leaflets: 4,9/7,2 mm

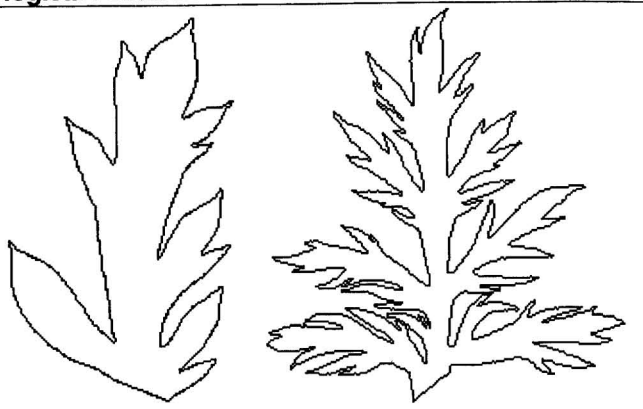
Width of the leaflets rachis: 0,75 mm

Width/length of involucrum: 23,3/4,5 mm

Width/length of ligule: 2,8/2,7 mm

Corolla tube length divided by ligule length: 0,69

Diameter of pollengrains (with spines): 37,6 µm

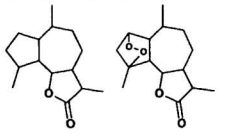


Chemical Features

GLC-characteristics: sabinene,ß-pinene, 1,8-cineol, camphore and borneol dominant.

Colour of glands: uncoloured or light green.

Sesquiterpene skeletons:



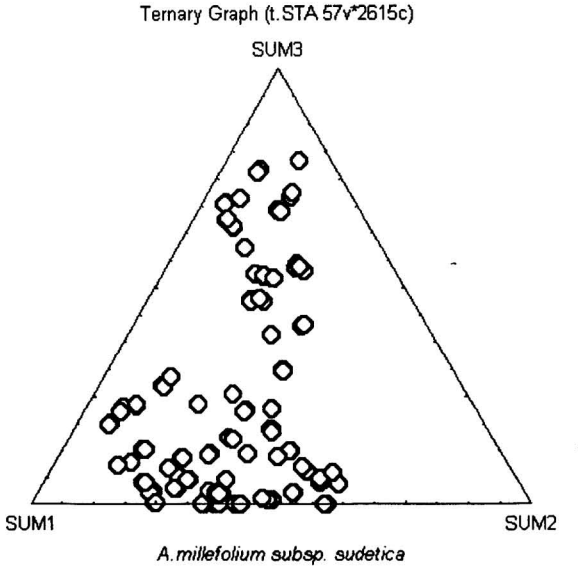


Table 2l. Short morphological, cytological and chemical characteristics of *A. millefolium* ssp. *sudetica*.

A. asplenifolia	**Proazulenes and other guaianolides***
8α-angeloxy-artabsin **1**	R_x = 1.67 AP blue, AS green
8α-tigloxy-artabsin **2**	R_x = 1.51 AP blue, AS green
achillicin **3**	R_x = 1.24 AP blue, AS green
4α-hydroxy-6α-angeloxy-9α-acetoxy-5αH,7αH,8βH, 11αH-guaia-1(10),2-dien-12,8-olide **4**	R_x = 1.06 AP blue, AS green
4α-hydroxy-6α-tigloxy-9α-acetoxy-5αH,7αH,8βH,11αH-guaia-1(10),2-dien-12,8-olide **5**	R_x = 1.06 AP blue, AS green
4α-hydroxy-6α,9α-diacetoxy-5αH,7αH,8βH,11αH-guaia-1(10),2-dien-12,8-olide **6**	R_x = 0.85 AP blue, AS green
4β-hydroxy-6α-angeloxy-9α-acetoxy-5αH,7αH,8βH, 11αH-guaia-1(10),2-dien-12,8-olide **7**	R_x = 1.13 AP blue, AS green
8-desacetyl-4-epi-matricin **8**	R_x = 0.31 AP blue, AS green
8-desacetyl-8-tigloyl-4-epi-matricin **9**	R_x = 1.24 AP blue, AS green
8α-angeloxy-3-oxa-artabsin **10**	R_x = 1.59 AP pink, AS violet
8α-tigloxy-3-oxa-artabsin **11**	R_x = 1.43 AP pink, AS violet
3-oxa-achillicin **12**	R_x = 1.16 AP pink, AS violet
8α-angeloxy-2α,4α,10β-trihydroxy-6βH,7αH,11βH-1(5)-guaien-12,6α-olide **13**	R_x = 0.25 AP blue, AS green
8α-angeloxy-1β,2β:4β,5β-diepoxy-10β-hydroxy-6βH,7αH,11βH-guaian-12,6α-olide **14**	R_x = 0.45 AP blue, AS green
8α-angeloxy-4α,10β-dihydroxy-2-oxo-6βH,7αH,11βH-1(5)-guaien-12,6α-olide **15**	R_x = 0.63 AP blue, AS green
Kastner *et al.*, 1992a (**4-8**), Schröder *et al.*, 1994 (**1-3,10-12**), Kubelka *et al.*, 1999 (**9**), Glasl *et al.*, 2001 (**13-15**)	
A. roseoalba	**Proazulenes and other guaianolides***
8α-angeloxy-artabsin **1**	R_x = 1.67 AP blue, AS green
8α-tigloxy-artabsin **2**	R_x = 1.51 AP blue, AS green
achillicin **3**	R_x = 1.24 AP blue, AS green
8-desacetyl-8-tigloyl-matricin **16**	R_x = 1.24 AP blue, AS green
8-desacetyl-8-tigloyl-4-epi-matricin **9**	R_x = 1.24 AP blue, AS green
8α-angeloxy-3-oxa-artabsin **10**	R_x = 1.59 AP pink, AS violet
8α-tigloxy-3-oxa-artabsin **11**	R_x = 1.43 AP pink, AS violet
3-oxa-achillicin **12**	R_x = 1.16 AP pink, AS violet
5α- hydroxy-8-desacetyl-8-tigloyl-matricarin **17**	R_x = 1.20 AP n.r. AS n.r. sometimes light pink, fl.qu.
8α-angeloxy-2α,4α,10β-trihydroxy-6βH,7αH,11βH-1(5)-guaien-12,6α-olide **13**	R_x = 0.25 AP blue, AS green
8α-angeloxy-1b,2b:4b,5b-diepoxy-10β-hydroxy-6βH,7αH,11βH-guaian-12,6α-olide **14**	R_x = 0.45 AP blue, AS green
8α-angeloxy-4α,10β-dihydroxy-2-oxo-6βH,7αH,11βH-1(5)-guaien-12,6α-olide **15**	R_x = 0.63 AP blue, AS green
Kastner *et al.*, 1991 (**1-3,9,16**), Kastner *et al.*, 1991a (**10-12, 17**), Glasl *et al.*, 2001 (**13-15**)	

Table 2. List of taxa of the *A. millefolium* group and *A. crithmifolia* including sesquiterpenes, R_x values, colours with spraying reagents and references.

A. collina	**Proazulenes and other guaianolides***
8α-angeloxy-artabsin **1**	R_x = 1.67 AP blue, AS green
8α-tigloxy-artabsin **2**	R_x = 1.51 AP blue, AS green
achillicin **3**	R_x = 1.24 AP blue, AS green
8-desacetyl-8-tigloyl-4-epi-matricin **9**	R_x = 1.24 AP blue, AS green
8α-angeloxy-3-oxa-artabsin **10**	R_x = 1.59 AP pink, AS violet
8α-tigloxy-3-oxa-artabsin **11**	R_x = 1.43 AP pink, AS violet
3-oxa-achillicin **12**	R_x = 1.16 AP pink, AS violet
matricarin **18**	R_x = 1.73 AP n.r. AS n.r. sometimes light pink, fl.qu.
8-desacetyl-matricarin **19**	R_x = 0.59 AP n.r. AS n.r. sometimes light pink, fl.qu.
8α-angeloxy-2α,4α,10β-trihydroxy-6βH,7αH,11βH-1(5)-guaien-12,6α-olide **13**	R_x = 0.25 AP blue, AS green
8α-angeloxy-1b,2b:4b,5b-diepoxy-10β-hydroxy-6βH,7αH,11βH-guaian-12,6α-olide **14**	R_x = 0.45 AP blue, AS green
8α-angeloxy-4α,10β-dihydroxy-2-oxo-6βH,7αH,11βH-1(5)-guaien-12,6α-olide **15**	R_x = 0.63 AP blue, AS green
Kastner *et al.*, 1991a (**10-12**), Kastner *et al.*, 1991b (**1-3**), Kubelka *et al.*, 1999 (**9, 18, 19**), Glasl *et al.*, 2001 (**13-15**)	
A. ceretanica	**Proazulenes and other guaianolides***
8α-angeloxy-artabsin **1**	R_x = 1.67 AP blue, AS green
8α-tigloxy-artabsin **2**	R_x = 1.51 AP blue, AS green
achillicin **3**	R_x = 1.24 AP blue, AS green
2α,8α -dihydroxy-1α,5α, 6ß, 11ßH-guaia-3,10(14)-dien-12,6-olide **20**	R_x = 0.16 AP blue, AS green
8α-acetoxy-2α-hydroxy-1α,5α, 6ß, 11ßH-guaia-3,10(14)-dien-12,6-olide **21**	R_x = 0.89 AP blue, AS green
8α-angeloxy-3-oxa-artabsin **10**	R_x = 1.59 AP pink, AS violet
8α-tigloxy-3-oxa-artabsin **11**	R_x = 1.43 AP pink, AS violet
3-oxa-achillicin **12**	R_x = 1.16 AP pink, AS violet
matricarin **18**	R_x = 1.73 AP n.r. AS n.r. sometimes light pink, fl.qu.
8-desacetyl-matricarin **19**	R_x = 0.95 AP n.r. AS n.r. sometimes light pink, fl.qu.
8α-angeloxy-2α,4α,10β-trihydroxy-6βH,7αH,11βH-1(5)-guaien-12,6α-olide **13**	R_x = 0.25 AP blue, AS green
8α-angeloxy-1b,2b:4b,5b-diepoxy-10β-hydroxy-6βH,7αH,11βH-guaian-12,6α-olide **14**	R_x = 0.45 AP blue, AS green
8α-angeloxy-4α,10β-dihydroxy-2-oxo-6βH,7αH,11βH-1(5)-guaien-12,6α-olide **15**	R_x = 0.63 AP blue, AS green
Glasl *et al.*, 1997 (**1-3,10-12,18,19**), Wawrosch *et al., 1997,* Glasl *et al.*, 1999 (**20,21**), Glasl *et al.*, 2001 (**13-15**)	

Table 2. (continued) List of taxa of the *A. millefolium* group and *A. crithmifolia* including sesquiterpenes, R_x values, colours with spraying reagents and references.

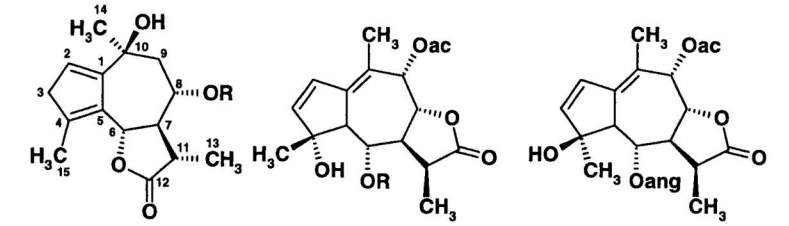
A. pannonica	**Different skeletons***
1,4-dihydroxy-germacra-5E-10(14)-diene **22**	R_x = 0.32 AP n.r., AS blue
11,13-dehydrodesacetylmatricarin **23**	R_x = 0.70 AP n.r. AS n.r. sometimes light pink, fl.qu.
α-longipin-2-en-1-on **24**	R_x = 1.95 AP n.r., AS orange, fl.qu.
(6E)-5-tigloxy-9-hydroxynerolidol **25**	R_x = 1.14 AP blue, AS dark blue
spathulenol **26**	R_x = 1.93 AP dark blue, AS violet
Sosa *et al.*, 2001 (**22**), Werner *et al.*, 2002 (**23-26**)	
A. pratensis	**Eudesmanolides***
tauremisin **27**	R_x = 0.97 AP + AS n.r., fl.qu.
arglanin **28**	R_x = 1.04 AP n.r., AS green, fl.qu.
4α-hydroperoxy-4α-dehydroxy-arglanin **29**	R_x = 1.07 AP yellow, AS orange
4-epi-arglanin **30**	R_x = 1.15 AP n.r., AS green, fl.qu.
santamarin **31**	R_x = 1.51 AP n.r., AS violet, fl.qu
Glasl *et al.*, 1995 (**27-31**)	
***A. millefolium* s.l.**	**Different Skeletons***
α-peroxyachifolid **32**	R_x = 1.79 AP green, AS brown
8α-angeloxyartabsin-1,4-endoperoxide **33**	R_x not indicated in Ref.
10α-detigloxy-10α-isovaleroxy-α-peroxyachifolid **34**	R_x not indicated in Ref.
isoapressin **35**	R_x not indicated in Ref.
8α-deacetoxy-8α-tigloxy-ezomontanin **36**	R_x = 1.74 AP green, AS brown
β-peroxyisoachifolid **37**	R_x not indicated in Ref.
isoachifolidien **38**	R_x not indicated in Ref.
santamarin **31**	R_x = 1.51 AP green, AS brown
1,4-dihydroxy-germacra-5E-10(14)-diene **22**	R_x = 0.32 AP n.r., AS blue
Cepak, 1997 (**32,36,31,22**), Rücker *et al.*, 1991 (**32, 37**), Hausen *et al.*, 1991 (**32-34, 36, 37**), Rücker *et al.*, 1992 (**38**), Rücker *et al.*, 1993 (**35**)	
A. millefolium* ssp. *sudetica	**Guaianolides***
desacetoxymatricarin **39**	R_x = 0.20 AP n.r. AS n.r. sometimes light pink
8α-tigloxy-1α,4α-epidioxy-10β-hydroxy-2,11(13)-guaiadien-12,6α-olide **40**	R_x = 1.07 AP bluegreen, AS brown
Haberl, 1999 (**39**), Kuert, 1999 (**40**)	

Table 2. (continued) List of taxa of the *A. millefolium* group and *A. crithmifolia* including sesquiterpenes, R_x values, colours with spraying reagents and references.

A. crithmifolia	**Different Skeletons***
rupicolin A **41**, rupicolin A acetate	R_x not indicated in Ref.
rupicolin B **42**, rupicolin B acetate	R_x not indicated in Ref.
1β, 10α-epoxy-3β,9β-diacetoxy-11α,13-dihydrocostunolide **47**	R_x not indicated in Ref.
desacetyl-1α,4α-dihydroxybishopsolicepolide **48**	R_x not indicated in Ref.
desacetyl-1α,4β-dihydroxybishopsolicepolide **49**	R_x not indicated in Ref.
1β,2β:3β,4β-diepoxy-8α,10α-dihydroxyguai-11(13)-en-12,6α-olide **50**	R_x not indicated in Ref.
1β,3β-dihydroxy-eudesma-4(15), 11(13)-dien-12,6α-olide **51**	R_x not indicated in Ref.
crithmifolide **52**	R_x not indicated in Ref.
acrifolide **53**	R_x not indicated in Ref.
Milosavljevic *et al.*, 1991 (**41, 42, 47**), Milosavljevic *et al.*, 1994 (**48-50**), Todorova *et al.*, 1998 (**51, 52**), Todorova *et al.*, 2000 (**53**)	
A. setacea	**Guaianolides***
rupicolin A **41**	R_x = 0.28 AP blue, AS green
rupicolin B **42**	R_x = 0.28 AP blue, AS green
11,13-dehydro-desacetylmatricarin **23**	R_x = 0.7 AP n.r. AS n.r. sometimes light pink
Zitterl-Eglseer *et al.*, 1991 (**41, 42, 23**)	
A. styriaca	**Different Skeletons***
5β-tigloyl-achillifolin **43**	R_x = 1.46 AP n.r., AS pink
α -longipin-2-en-1-on **24**	R_x = 1.95 AP n.r., AS orange, fl.qu.
10β-hydroxy-α-longipin-2-en-1-on **44**	R_x = 0.79 AP n.r., AS orange, fl.qu.
arglanin **28**	R_x = 1.04 AP n.r., AS green, fl.qu.
arglanin-4-methylether **45**	R_x = 0.87 AP n.r., AS green
tauremisin **27**	R_x = 0.97 AP n.r., AS n.r., fl.qu.
3-eudesmen-1β,6α,11-triol **46**	R_x = 0.35 AP n.r., AS violet
Kastner *et al.*, 1996 (**43, 44, 24**), Stöckelmayer, 1998 (**27, 28, 45, 46**)	

* The bold numbers correspond to the formulas shown in fig. 5
R_x values calculated with matricin as reference (Rf = 0.4), mobile phase: dichloromethane-acetone (9+1)
AP: Acetic acid – phosphoric acid – reagent (Stahl, 1967)
AS: Anisaldehyde – sulphuric acid – reagent (Dequeker, 1964)
n.r.: nor reaction with spraying reagent
fl.qu.: fluorescence quenching (UV_{254})

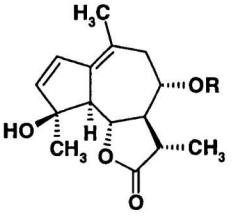
Table 2. (continued) List of taxa of the *A. millefolium* group and *A. crithmifolia* including sesquiterpenes, R_x values, colours with spraying reagents and references.



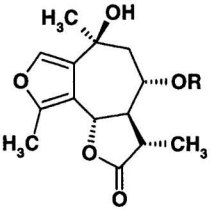
1: R=angeloyl
2: R=tigloyl
3: R=acetyl

4: R=angeloyl
5: R=tigloyl
6: R=acetyl

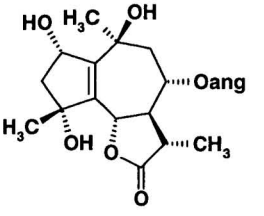
7



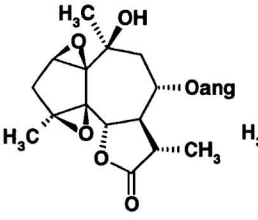
8: R=H
9: R=tigloyl



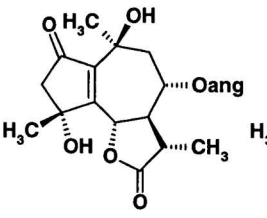
10: R= angeloyl
11: R= tigloyl
12: R= acetyl



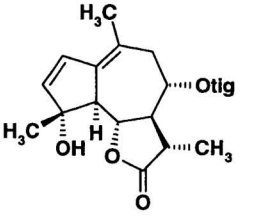
13



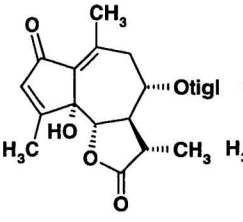
14



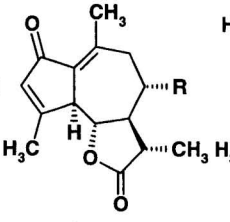
15



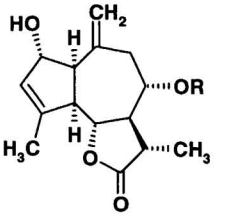
16



17



18: R= acetoxy
19: R= OH
39: R= H



20: R= H
21: R= acetyl

Figure 5. Sesquiterpenes of taxa of the *A. millefolium* group and *A. crithmifolia*.

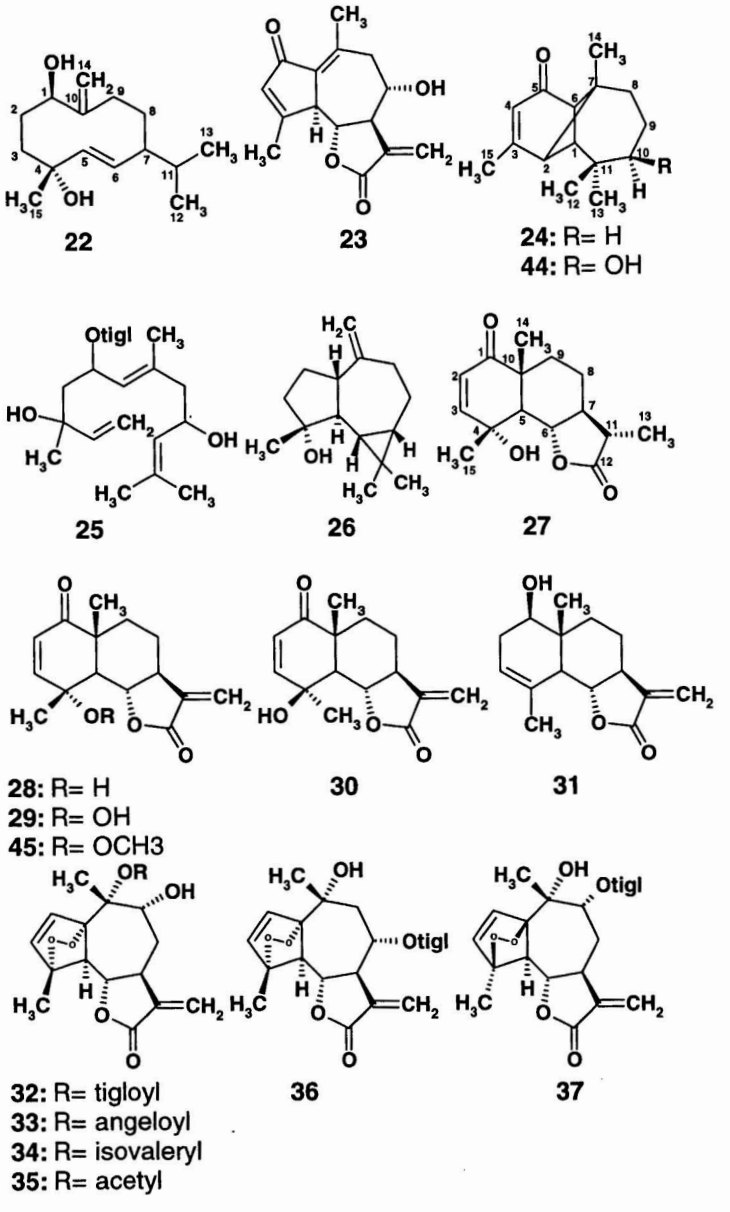


Figure 5. (continued) Sesquiterpenes of taxa of the *A. millefolium* group and *A. crithmifolia*.

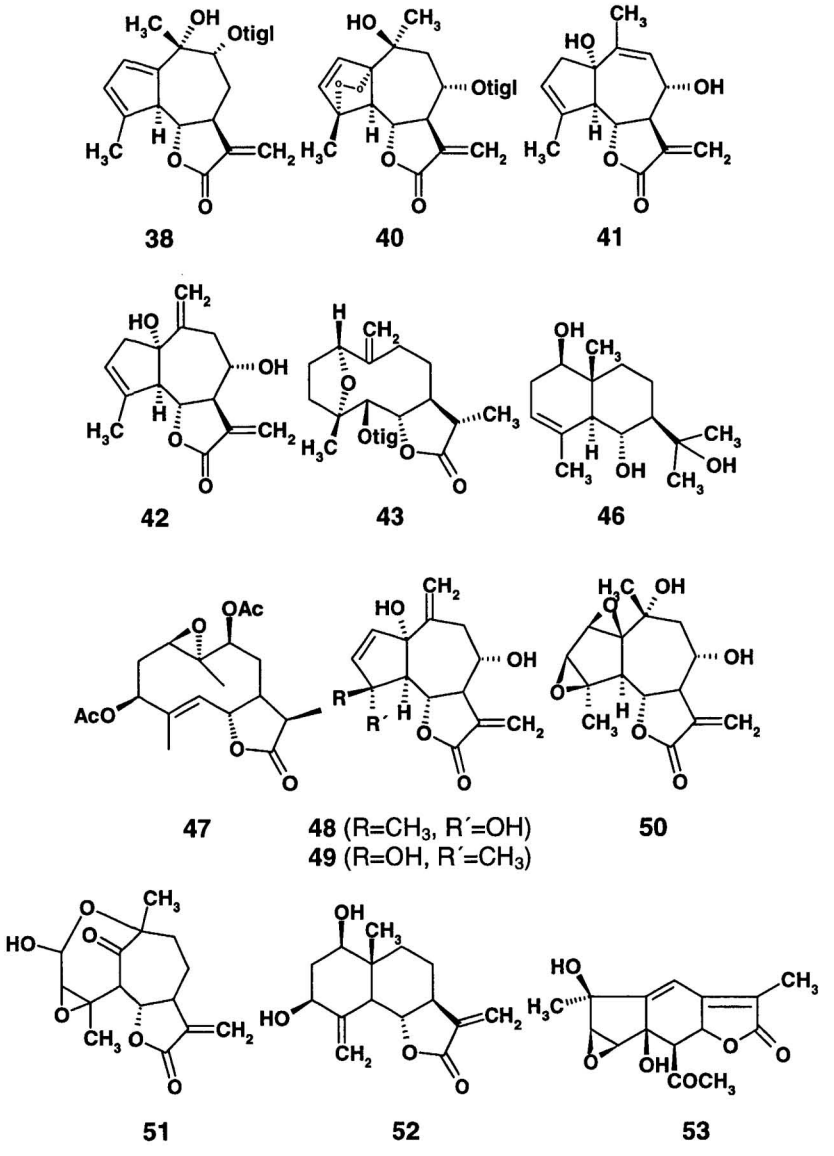


Figure 5. (continued) Sesquiterpenes of taxa of the *A. millefolium* group and *A. crithmifolia*.

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