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CONTRIBUTION TO THE CHEMOTAXONOMY OF SOME EUROPEAN *PETASITES* SPECIES

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Abstract—Thin-layer chromatography of certain chemical components of some species of *Petasites* can be used for quick and reliable identification of non-flowering individuals and interspecific hybrids. The substances are: furanopetasin in *P. hybridus* (L.) Gaertn., Meyer et Scherb., petasalbin in *P. albus* (L.) Gaertn., and kablicin in *P. kablikianus* Tausch. ex Bercht. The phylogenetic position of *P. kablikianus* is discussed and it is concluded that this taxon should be considered as an independent species of hybrid origin, which underwent a speciation process of its own. Evidence is presented concerning the occurrence of two chemovars for *P. hybridus* and *P. albus*.

The genus *Petasites*, which belongs to the Asteraceae, is interesting but difficult to study. If we wish to account the complicated evolution of the Asteraceae it is very often difficult to decide whether or not a genus belongs to a highly specialized branch, to a phylogenetically young but still developing branch or to one established early and characterized by numerous reductions. For this reason we have recently taken advantage of the collaboration of botanists and chemists to enlarge the criteria for classification.

The occurrence of sesquiterpenoid lactones (compounds of the santonine, guaianolide, ambrosanolide, germacranolide and eremophilanolide type) may be taken for a new chemotaxonomic character. An appreciable number of these lactones have been studied in our laboratory (for review see Ref. 1), and a systematic study of the chemical components in representatives of the Asteraceae revealed the occurrence of sesquiterpenoid substances of the less usual eremophilane type. These compounds can also be used as an important distinguishing character of the taxa of the *Petasites*. Their structure and occurrence permit a better evaluation of morphological variability, and could also contribute to the elucidation of the phylogenetic relationship in the *Petasites*.

The sesquiterpenoid compounds of the eremophilane series were first found by two Swiss groups [2,3] in the rhizomes of *P. hybridus* (L.) Gaertn., Meyer et Scherb. They isolated substances of the petasine type, the latter being an unsaturated hydroxyketone esterified with angelic acid (I). However, in our laboratory, rhizomes of this species were found to contain compounds derived from furoeremophilane (II) or from the biogenetically related eremophilenolide (III). We have so far isolated from the taxa of the *Petasites* studied, more than fifteen substances of this basic type and determined their structure[4] (Table 1).

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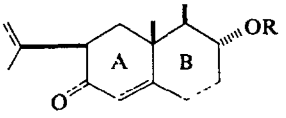
[1] F. Šorm and L. Dolejš, *Guaianolides and Germacranolides*. Hermann, Paris (1965).

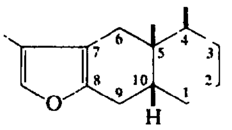
[2] A. Stoll, R. Morf, A. Rheiner and J. Renz, *Experientia* **12**, 360 (1956).

[3] A. Aebi, J. Büchi, T. Waaler, E. Eichenberger and J. Smitz, *Pharm. Acta Helv*. **30**, 277 (1955).

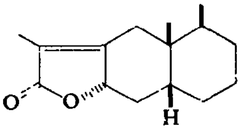
[4] L. Novotný and F. Šorm, In *Beiträge zur Biochemie und Physiologie von Naturstoffen*, p. 327. Festschrift Mothes K., VEB g. Fischer Verlag, Jena (1965).



(I) Petasine, R = Angelyl



(II) Furoeremophilane



(III) Eremophilenolide

TABLE 1

Species and compound	Formula	M.P. (°C)	$[\alpha]_D^{20}$ (°)
P. hybridus			
Furanoeremophilane	$C_{15}H_{22}O$	—	− 8·4
Furanoeremophilane	$C_{15}H_{20}O_2$	150	± 0
Furanopetasin	$C_{20}H_{28}O_4$	105–106	± 0
Eremophilenolide	$C_{15}H_{22}O_2$	125	+ 16·6
Petasitolide A	$C_{20}H_{28}O_4$	147	+ 48
Petasitolide	$C_{20}H_{28}O_4$	146	+ 31·8
S-Petasitolide A	$C_{19}H_{26}O_4$	201–202	− 15·3
S-Petasitolide B	$C_{19}H_{26}O_4$	199–200	− 32·9
Dimethoxydihydro-furanoeremophilane	$C_{17}H_{28}O_3$	103	+ 66·3
P. albus			
Petasalbin	$C_{15}H_{22}O_2$	81–82	− 11·2
Albopetasin	$C_{20}H_{28}O_3$	106–107	−137
Albopetasol	$C_{15}H_{22}O_3$	178–180	− 24·1
6-Hydroxyeremophilenolide	$C_{15}H_{22}O_3$	208	+207·4
P. kablikianus			
Kablicin	$C_{20}H_{28}O_5$	—	− 18·5
P. hybridus, *P. albus* and *P. kablikianus*			
Eremophilene	$C_{15}H_{24}$	—	−104·2
P. albus and *P. kablikianus*			
2-Angelylfuroeremophilane	$C_{15}H_{22}O_3$	—	− 3·7
P. albus, *P. kablikianus*, *P. japonicus* and *P. spurius*			
Angelyljaponicin	$C_{20}H_{28}O_4$	—	− 21·5
Diangelyljaponicin	$C_{25}H_{34}O_5$	—	− 5·2

The analytical method employed for surveying the occurrence of these compounds is based on thin-layer chromatography on silica gel according to Stahl,[5] which was used earlier

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[5] E. STAHL, *Dünschicht-Chromatographie.* Springer Verlag (1965).

for the analysis of substances of this type in extracts.[6] The advantage of this method consists in the rapid determination of components in unpurified light petroleum extracts of dried rhizomes or methanolic extracts of fresh samples (see Experimental). The position of the compounds on the chromatograms was detected by spraying with concentrated sulphuric acid and gentle warming. Under such conditions coloured spots are formed, which were identified by comparing their R_f values with those of authentic specimens (Table 2). It is worth mentioning that substances oxidized at C_6 of the furoeremophilane skeleton give a strong red to purple coloration; the same is true for substances carrying a hydroxyl group on the B ring (see I), or for the parent furoeremophilane (II). On the other hand, substances oxidized at C_9 give a typical dark-green coloration (furanopetasin and kablicin). The reaction is very sensitive and the whole analysis can be carried out with a small fragment of rhizome.

TABLE 2. R_f VALUES OF REFERENCE COMPOUNDS USED FOR CHEMOTAXONOMIC DIFFERENTIATION OF SOME *Petasites* SPECIES AND INTERSPECIFIC HYBRIDS

Compound	R_f*	Colour†
Kablicin	0·11	Deep green
Angelyljaponicin	0·17	Red
Furanopetasin	0·28	Green
Petasalbin	0·63	Red–purple
Diangelyljaponicin	0·84	Red

* On silica gel in dichloromethane.
† After spraying conc. H_2SO_4 and heating.

It is important to point out that the substances of the eremophilane type have been rarely found in nature until now, and that their occurrence was observed for the first time (eremophilone and its derivatives) in the tropical species *Eremophila mitchelli* Benth.[7] (Myoporaceae). Of course, eremophilane derivatives in the Asteraceae are not confined only to the *Petasites*, the occurrence of petasalbin and of its dehydroderivatives ligularone having been demonstrated also in the *Ligularia sibirica* Cass.[8] They also occur in *Euryops floribundus* N.E.Br.[9]

The genera *Ligularia*, *Euryops* and *Petasites* are often arranged from the morphological point of view in different taxa at the level of tribe or subtribe. The fact that eremophilane derivatives occur in representatives of all three genera shows the possibility of putting them into the one tribe Senecioneae.

Intrageneric relationships probably may be evaluated on the grounds that furanopetasin, petasalbin and kablicin can all be possibly formed from one hypothetical precursor by oxidation or substitution.[10] Unfortunately, the fact that the chemical components of the species *P. albus* (L.) Gaertn., *P. japonicus* (Sieb. et Zucc.) Maxim. subsp. *giganteus* Kitam. and *P. spurius* (Retz.) Reichenb. are practically identical cannot be utilized immediately for taxonomic purposes. From the morphological point of view, these species represent rather

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[6] L. NOVOTNÝ, Z. SAMEK, V. HEROUT and F. ŠORM, *Colletion Czech. Chem. Commun.* (in press).
[7] A. E. BRADFIELD, A. R. PENFOLD and J. L. SIMONSEN, *J. Chem. Soc.* 2744 (1932); 767 (1938); 60 (1941).
[8] K. TAKEDA (personal communication).
[9] D. E. A. RIVETT (personal communication).
[10] R. E. ALSTON and B. L. TURNER, *Biochemical Systematics*. Prentice-Hall, Englewood Cliffs, New Jersey (1963)

distant taxa and they were classified into separate sections or series in the proposed system of the *Petasites*; recently Kupriyanova[11] has also treated these species analogously.

The fundamental furoeremophilane skeleton of these compounds, present also in compounds from different species, is oxidized in the ring A either at C_6 or C_9. If derivatives oxidized at C_6 were found only in three from the four mentioned taxa, and if in the *P. hybridus* compounds only oxidized at C_9 were found, then this fact cannot be considered as a manifestation of a closer or a more distant relationship. It can, however, point to the degree of organization reached by these taxa. For example, we can envisage an equal degree of specialization, attained in several parallel lines. However, morphogenetic studies which we made previously do not permit a generalization in this respect either. In *P. spurius* we found a different route of the supposed development of styles to that in *P. japonicus* and *P. hybridus*. On the other hand, according to our views, *P. spurius* represents the first member in the evolutionary line of the ligulate taxa (subgen. Nardosmia), the other species (*P. hybridus*, *P. japonicus* and *P. albus*) belong to the line of aligulate taxons (subgen. Petasites).

The difference in the position of the oxidized groups in the compounds is, however, a utilizable character in the evaluation of the relationships of *P. kablikianus* Tausch ex Bercht. This taxon comprises eremophilane derivatives oxidized at C_6 (angelyl and diangelyljaponicine) as well as at the carbon atom 9 (kablicin). Hence, in the case of this taxon we can speak about a distinct hybrid effect, because possible parent species—*P. hybridus* and *P. albus*—are members of different types, as determined by the position of the oxidized group.

The divergent views on the actual status of *P. kablikianus* are almost as old as the discovery and description of the taxon itself. However, the early views concerning hybrid origin have several times been refuted, because the true hybrid between *P. albus* and *P. hybridus* has been discovered many times.[12,13] The difference of this hybrid from *P. kablikianus* is evident not only from morphological characters, but also from the absence of kablicin which is strongly present in *P. kablikianus*. Kablicin is as characteristic of the latter species as is petasalbin for *P. albus* and furanopetasin for *P. hybridus*. The true hybrid of *P. albus* and *P. hybridus* (= *P.* × *rechingeri*) has both compounds—petasalbin and furopetasin, but kablicin never occurs.

P. kablikianus can thus be considered as a taxon of a similar systematical category as for example the taxa *P. albus* or *P. hybridus*, i.e. as an independent species defined, in addition to its morphological features and its area of distribution,[13,14,15] by the occurrence of a specific compound—kablicin. Hence, it is preferable not to classify it as an intraspecific taxon of one of the two species, although it is at present sometimes classified in this way (*P. albus* subsp. *kablikianus* Löve et Löve[15]).

P. kablikianus is not an actual F_1-hybrid of the other two species, because the proper hybrid, *P.* × *rechingeri* Hayek (*P. albus* × *P. hybridus*) can originate in nature only after overcoming the cytogenetic and phenological barrier, not mentioning morphological and ecological differences.

P. kablikianus is, however, most probably of hybrid origin. On the basis of morphological studies we found it has three groups of characters: intermediate characters, characters identical with those of one or the other of the supposed parents, and finally, completely intrinsic, new characters. In the first two groups can be placed the occurrence of substances

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[11] L. A. KUPRIYANOVA, In B. K. SHISHKIN and E. G. BOBROV: *Flora SSSR* **26**, 642 (1961).

[12] J. TOMAN and F. STARÝ, *Preslia* **37**, 264 (1965).

[13] J. TOMAN and F. STARÝ, *Preslia* **38**, 168 (1966).

[14] J. ŠOUREK, *Rozpravy Česk. Akad. Věd* **72**, 65 (1962).

[15] A. LÖVE and D. LÖVE, *Opera Botan.* **5**, 347 (1961).

with an oxygenated group at C_6 and C_9. In the last-mentioned group, in addition to the form and the size of the basal sinus of the leaf, the shape and the size of the head, glands, involucre bracts and pollen grains, we can also consider the occurrence of the specific component—kablicin (see Fig. 1).

We can conclude therefore that *P. kablikianus* is a species of hybrid origin. If this is so, for *P. kablikianus* on the basis of morphological and chemical evidence, then the same is true for *P. paradoxus* (Retz.) Baum. on the basis of purely morphological studies. The proposed chemical analysis will show whether the morphological and genetical relationships shown in Fig. 1 are correct, or whether it will be necessary to interpret in another way this Alpine parallel of the Krkonoše-Carpatien species of *P. kablikianus*.

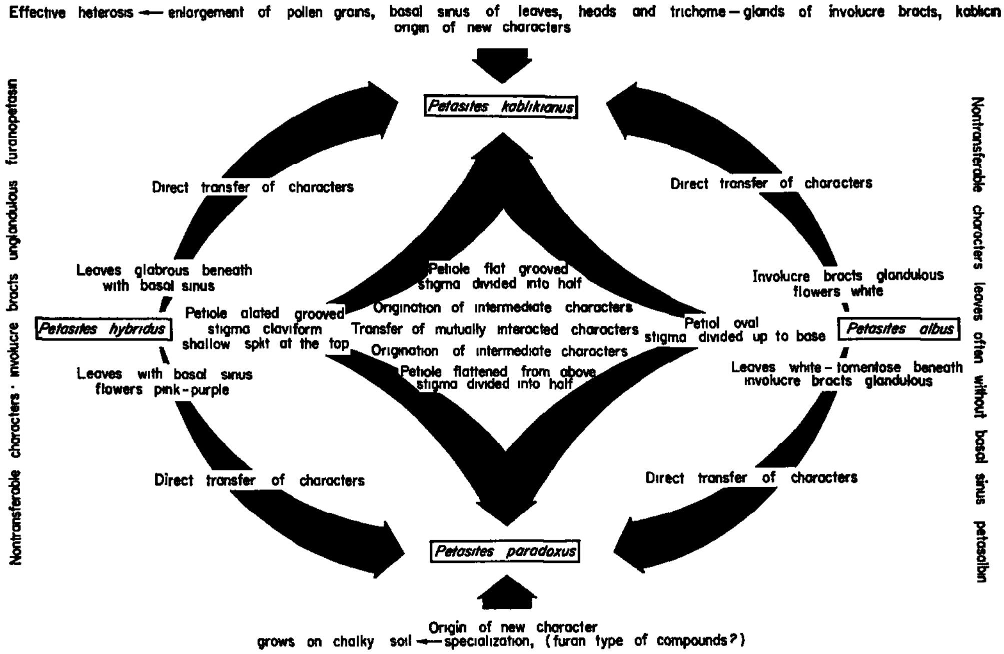

FIG. 1. MORPHOLOGICAL RELATIONSHIP OF THE FOUR CENTRAL EUROPEAN SPECIES OF THE GENUS *Petasites*.

Out of these we consider *P. hybridus* and *P. albus* as post-tertiary parental species and *P. kablikianus* (from High Sudete and Carpation regions) and parallel species *P. paradoxus* (from Alpine-Dinaric region) as derived species, presumably of hybrid origin.

As we have already mentioned above, furanopetasin and petasalbin are always found together in hybrids between *P. albus* and *P. hybridus* and so the presence of both substances in a specimen may be taken to prove unambiguously that it is an actual hybrid. Even when determination based on morphological features does not indicate a natural hybrid, a more detailed study of such specimens confirmed the chemical analysis in all instances. Similarly, the occurrence of kablicin together with petasalbin in specimens showing many striking features characteristic of *P. albus* indicated in all cases the probable hybrid nature (*P. albus* × *kablikianus*) by less striking characters inherited from *P. kablikianus*.

Such analyses were carried out with material from the original localities, as well as with samples cultivated in experimental gardens. The study of the same specimen allows us to follow the development of all morphological and micro-morphological characters during the whole vegetation period; a possible error, which could arise by collecting an insufficient amount of herbarium samples, is thus avoided.

As the morphological identification of hybrids corresponded in all instances with the supposed combinations of furoeremophilane derivatives, we consider that the determination of these substances represents a very reliable criterion of interspecific hybridization.

As the species *P. albus* and *P. hybridus* grow over almost two-thirds of Europe it should not be surprising to find variations in the content of the above-mentioned compounds in samples from various localities. We were successful in obtaining samples unambiguously agreeing morphologically with *P. hybridus*, which contained, however, eremophilane derivatives of the petasin type, instead of the analogous derivatives of the furan type. We recently obtained a collection of fifteen samples from different localities in western Europe* which has shown that eight of these samples were of petasin type (e.g. (I)) and the remaining seven samples belonged to the furan type (e.g. (II)) (see Table 3).

TABLE 3. LIST OF LOCALITIES OF *P. hybridus* AND INDICATION OF THE CHEMOVAR TYPE

Locality	Chemovar
Botanical garden, Münster	Petasin
Krkonoše, junction of the rivers Úpa and Úpice	Petasin
Near Orbey, Vogesen	Furan
Near Lay St. Christophe, northwards from Nancy, France	Petasin
At Westhalten, near Colmar, France	Petasin
Gudingen (Saar, near Saarbrücken)	Petasin
Alsbach, near Marpingen, Saarland	Furan
Aartal, near Hohenstein (Taunus)	Petasin
Near Oberweissenbrunn, northwards from Gersfeld (Rhön)	Furan
Vallée de Ernz Noire (Mullerthal), Luxemburg	Furan
Rurtal, near Hammer, eastwards from Monschau (Eifel)	Furan
Near Holzminden	Furan
Hartmannshof (eastwards from Nürnberg)	Petasin
Großschwindau, north-eastwards from München	Petasin
Wasserburg, eastwards from München	Petasin
Unter Kainisch (Trauntal)	Petasin
Hollenburg, southwards from Klagenfurth	Furan

It can be inferred from the present results that *P. hybridus* exists in two chemovars, i.e. chemovar petasin and chemovar furan. In view of the great number of occurrences, it seems very probable that the chemovar petasin is not geographically demarcated. The existence of both chemovars in *P. hybridus* species corresponds very well with the morphological variability of this species. However, it was not possible hitherto to demonstrate that the occurrence of a certain type of constituents is restricted exclusively to some of the known intraspecific taxa.

P. albus is morphologically polytypic too, but rather less variable than *P. hybridus*. We were therefore not surprised to find in two localities a chemovar analogous to the petasin chemovar, i.e. a chemovar not containing furan derivatives.

We believe, therefore, that the chemovars differ in the content of their enzymatic systems.

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* We are very grateful to Professor Egon Stahl, Pharmakognostisches Institut der Universität des Saarlandes, Saarbrücken, for the provision of this collection.

The "petasin chemovar" evidently lacks the enzymes necessary for the oxidation of eremophilane derivatives to substances of the furanoeremophilane type, characteristic for the "furan chemovar". Further study of the enzyme systems in these chemovars, and similar investigations in other species of the genus *Petasites*, will be directed towards determination of enzyme activities, which should support these hypotheses. The extension of studies to other species and the intensification of morphogenetic studies concerning the origin and the development of ligules in the subgenus Nardosmia (Cass.) Nyman should enable us to evaluate in a better way the phylogenetic relationships within the genus *Petasites*.

EXPERIMENTAL

Preparation of the extract. (*a*) The washed rhizomes (15–50 g) were dried and ground to a powder and extracted with light petroleum (b.p. 40–60°) in the cold. The extract was concentrated by distillation and the residue dissolved in a tenfold amount of benzene, transferred to a brown vial, sealed under nitrogen, and stored in an ice-box. (*b*) Fresh rhizomes (10–15 g) were cut to small pieces and macerated with 100 ml of pure methanol in a closed vessel for 24 hr. The methanolic extract was filtered and diluted with water and extracted three times with 50 ml benzene. The benzene solution was dried (Na_2SO_4) and distilled under reduced pressure. The thick residue was diluted with dry benzene so as to bring the final concentration to approx. 10 per cent, and then stored as described under (*a*).

Thin-layer chromatography of extracts was carried out on silica gel* standard plates (20 × 20 cm) with the S-separation system according to Stahl.† Dichloromethane was used as solvent in multiple chromatography. Detection was carried out by spraying the dry plates with concentrated sulphuric acid and heating.

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* Kieselgel G according to Stahl, Merck, Darmstadt.

† From C. Desaga GmbH Company, Nachf. Erich Fecht, 69, Heidelberg 1.