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TERPENOIDS OF THE *PETASITES PARADOXUS* AND *PETASITES KABLIKIANUS* IN RELATION TO THEIR PHYLOGENY

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Abstract—In addition to a hydrocarbon fraction identical with those of other *Petasites* species, the light petroleum extract of *Petasites paradoxus* contained: kablicin, angelyljaponicin, kablikopetasin, furoeremophilane and a hitherto unidentified triterpenic alcohol $C_{30}H_{50}O$. These substances are identical with those encountered in *Petasites kablikianus*. The assumed parallel phylogenetic origin of both species, and their hybrid origin from the same parent taxa, seems to be confirmed.

IN A RECENT paper[1] we have dealt with chemotaxonomic problems of the most common European *Petasites* species: *P. hybridus* (L.) Gaertn., Mey. et Scherb., *P. albus* (L.) J. Gaertn. and *P. kablikianus* Tausch. ex Bercht. The most widely distributed, occurring over two-thirds of the European continent, is *P. hybridus* which grows from the lowlands to the mountains. A narrower area in central Europe is occupied by the submontane to subalpine species *P. albus*, whilst *P. kablikianus* is a montane to subalpine species which occupies an area which is oropolydisjunctive in the mountains of High-Sudeten, Carpathia and Illyria. We have compared the substances isolated from the rhizomes of *P. paradoxus* (Retz.) Baumg. which is a montane to alpine species with its centre in the Alps (the problem of the Pyrenean clone has not been satisfactorily solved) with the components of the above-mentioned species.

We ascertained earlier that each *Petasites* species contains typical components. In the case of *P. hybridus* it is furanopetasin (I) and 9-hydroxyfuroeremophilane (II),* in *P. albus* it is petasalbin (III) and angelyljaponicin (IV) while *P. kablikianus* contains kablicin (V) as the main component.

From the formulae of these compounds (Fig. 1) it can be seen that the main components characteristic of *P. hybridus* are hydroxylated at the position 9 (I and II), while in *P. albus* both substances contain hydroxy-groups at C_6 (III and IV). Both species must contain, therefore, specific enzymes enabling the oxidation of the basic furoeremophilane skeleton at these positions. In contrast to this the main component of *P. kablikianus*, kablicin (V), is oxidized both at position 9 as well as at position 6, which means that *P. kablikianus* evidently contains specific enzymes of both types.

This agrees well with the assumed phylogenetic origin of this species. We have already given a scheme of the relations of morphological characters of the species studied[1] and tried to demonstrate the hybrid origin of *P. kablikianus*. In our opinion it originated from a

* This substance was found in the light petroleum extract of the *P. hybridus* rhizomes only recently.[2] Its content is not constant and depends on the harvesting time.

[1] L. NOVOTNÝ, J. TOMAN, F. STARÝ, A. D. MARQUEZ, V. HEROUT and F. ŠORM, *Phytochem.* **5**, 1281 (1966).
[2] L. NOVOTNÝ, Z. SAMEK, J. HARMATHA and F. ŠORM, *Collection Czech. Chem. Commun.*, in press.

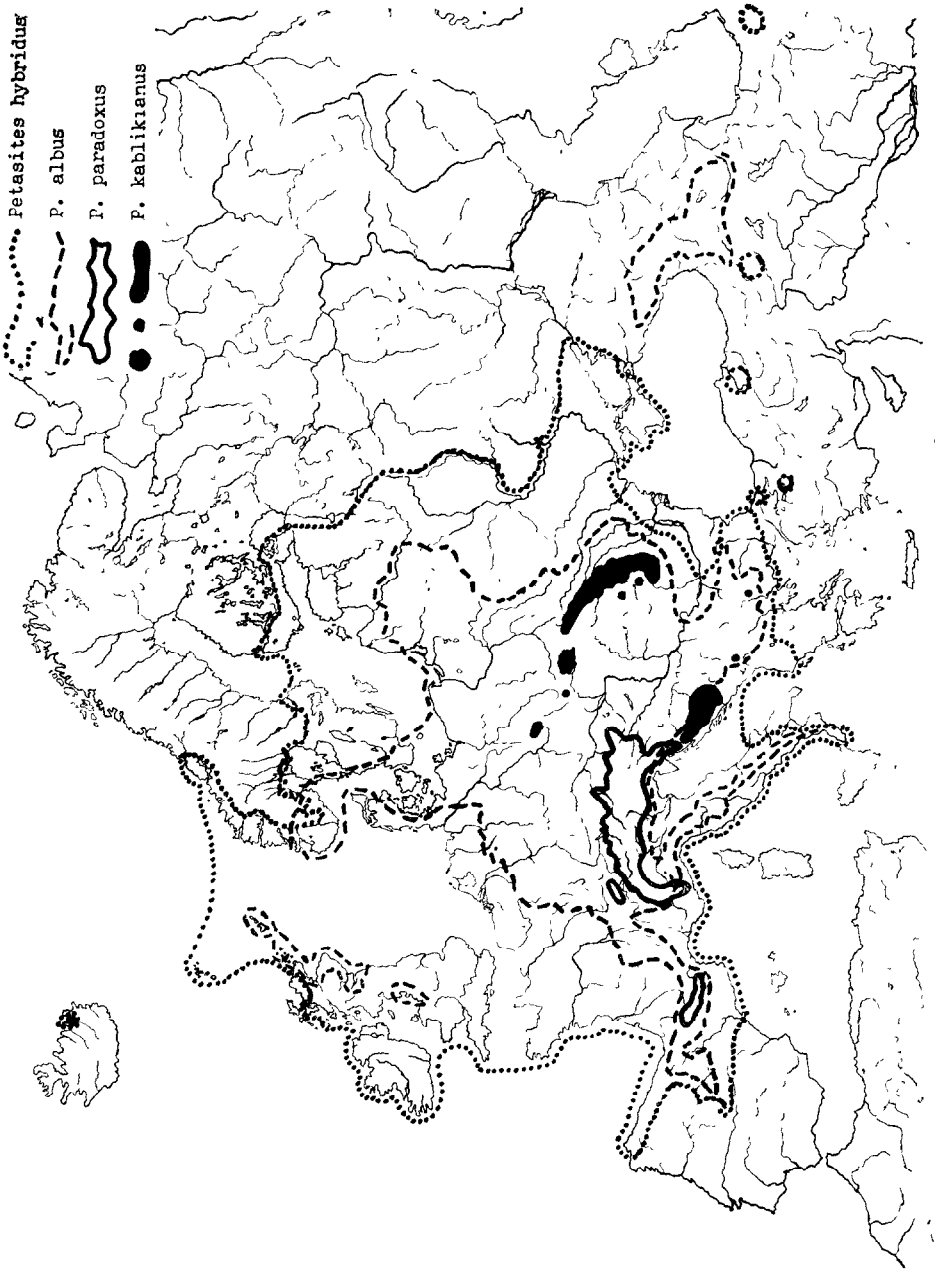
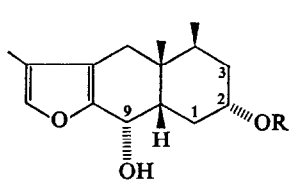
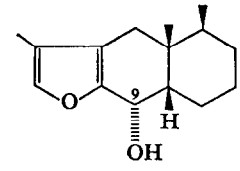


FIG 1 DISTRIBUTION OF THE STUDIED PETASITES SPECIES IN EUROPE.

glacial hybrid between *P. albus* and *P. hybridus*. We envisaged an analogous hybrid origin for *P. paradoxus*, at that time, of course, on morphological grounds only.



(I)



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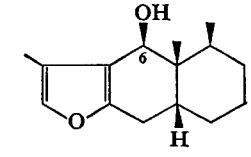
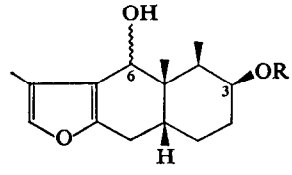
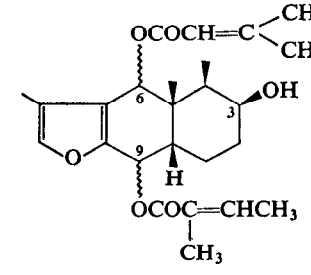


(III)



(IV)



(V)

In this paper we present our results concerning the compounds present in the light petroleum extract which have verified to the full extent our original suppositions. The hydrocarbon fraction agrees well with those from *P. albus*,[3] *P. hybridus*,[4] *P. spurius*,[5] *P. japonicus*,[6] *P. kablikianus*[7] and *Adenostyles alliarieae*,[8] with the characteristic presence of cremophilene and albene. In addition to the hitherto unidentified triterpenic alcohol $C_{30}H_{50}O$, m.p. 184°, we have also isolated angelyljaponicin (IV), furoeremophilane (VI), kablikopetasin (VII) and—which seems to be surprising at first sight—kablicin (V). When the results of this chemical analysis are compared with the scheme of morphological relationship[1] (where we concluded that *P. paradoxus* was an analogous parallel of *P. kablikianus*), the occurrence of the substances found seems logical. The occurrence of kablicin in these Sudetic-Carpathian and Alpine species strongly indicates their probable identical hybrid origin. We have no reason for a contrary opinion on the origin of *P. hybridus* and *P. albus* from *P. kablikianus* and *P. paradoxus*.

The origin of both *P. paradoxus* and *P. kablikianus* in a similar manner was possible, perhaps, because at the end (or perhaps during the course) of the last glaciation, bioclimatic changes took place in the Alpine-Carpathian montane system, which allowed the cytogenetic and ecological barriers typical of the parent species *P. hybridus* and *P. albus* to be overcome. From the hybrid populations formed by crossing these species, certain populations became fixed at various localities, being especially vigorous and with a genetical disposition for further evolution. In recent primary hybrids a relatively high percentage of well-germinating achenes can be found, and the plants possess a striking ability of vegetative spreading by

[3] J. HOCHMANNOVÁ, L. NOVOTNÝ and V. HEROUT, *Collection Czech. Chem. Commun.* **27**, 2711 (1962).
[4] J. HOCHMANNOVÁ, L. NOVOTNÝ and V. HEROUT, *Collection Czech. Chem. Commun.* **27**, 1870 (1962).
[5] L. NOVOTNÝ and V. HEROUT, *Collection Czech. Chem. Commun.* **27**, 2462 (1962).
[6] L. NOVOTNÝ and V. HEROUT, *Collection Czech. Chem. Commun.* **30**, 3579 (1965).
[7] L. NOVOTNÝ, M. SUCHÝ, A. D. MARQUEZ and F. ŠORM, *Collection Czech. Chem. Commun.*, in press.
[8] J. HARMATHA, L. NOVOTNÝ, V. HEROUT and F. ŠORM, *Collection Czech. Chem. Commun.*, in press.

their rhizomes. Thus, owing to the possiblity of inbreeding among the hybrids and the parent species, individuals (or whole populations) could split off representing the initial material for speciation. The completion of this process during the postglacial period was then perhaps characterized by fixation of heterosis in *P. kablikianus* and by adaptation to limestone[1] in *P. paradoxus*.

Three types of character transfer are possible: (a) Transfer types of character from one parent. (b) Formation of a character where that particular character occurs only in one parent and where the absence of that character in the second parent influences the transfer of the character only quantitatively but a qualitatively different intermediary character is not formed. (c) A true intermediary character is formed. Both species, i.e. *P. kablikianus* and *P. paradoxus* contain (keep) all these types. The enzymes which catalyse the oxidation of the basic furoeremophilane skeleton to kablicin must also be present in both species.

The manner of hydroxylation of this terpene can be most easily interpreted by mutual genetic influence of both parent species, i.e. *P. hybridus* and *P. albus*, during the crossing process. The fact that both parallel species *P. kablikianus* and *P. paradoxus* cross very easily with both original species *P. albus* and *P. hybridus*, while the latter give primary hybrids only very rarely, is merely the consequence of the relations described above, and also an indirect proof of them.

In these primary hybrids (*P.* × *rechingeri* Hayek) the enzymes do not influence one another, and thus only furanopetasin and petasalbin can be detected in the rhizomes of such hybrid (*P.* × *rechingeri* = *P. albus* × *P. hybridus*). On the contrary the formation of kablicin supposes a chromosomal *inter*action.

The analysis of the morphological characters of taxa of *Petasites* genus from North America—which is being completed—confirms the process of fixation of hybrid populations and of the formation of new taxa which is taking place on the American continent up to today but which has been completed in Europe and Asia for a long time. However, the value of this hypothesis has to be demonstrated by the identification of the corresponding chemical components.

EXPERIMENTAL

The dried rhizomes of *Petasites paradoxus* of three different sources* were ground and extracted with light petroleum. TLC on silica gel with light petroleum, ether, and benzene showed that the components of all three samples were identical. They were pooled therefore and concentrated. From 450 g (total weight) of rhizomes, 21 g of a thick oily extract was obtained, which was chromatographed on a neutral alumina column, act. III (830 g). The course of the chromatography is represented in Table 1.

The Hydrocarbon Fraction

Fractions 1–3 from the chromatography of the light petroleum extract were pooled and rechromatographed on alkaline alumina (act. I–II; 50 g). Elution with light petroleum gave a mixture of hydrocarbons which was analysed by gas–liquid chromatography. The character of the chromatogram was identical as in the case of other *Petasites* characterized by a typical occurrence of eremophilene and albene.[3–8]

Furoeremophilane

Elution with benzene of the above-mentioned chromatogram gave furoeremophilane, which was distilled and then identified by comparing its i.r. spectra and optical rotation with those of an authentic sample.[3]

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TABLE 1.

Fraction	Solvent	ml	Weight	Remark
1	Light petroleum	100	0·75	Mixture of hydrocarbons
2–3	Light petroleum	200	0·40	Hydrocarbons and furoeremophilane
4–5	Light petroleum	200	0·70	—
6	Benzene	100	0·90	Cryst. substance, m.p. 184°
7–10	Benzene	500	5·70	Angelyljaponicin and kablikopetasin
11–13	Benzene +2% EtOH	300	11·50	Kablicin
14	EtOH	200	2·1	Non-crystalizing oils

Triterpene $C_{30}H_{50}O$

After the concentration of *fraction* 6, a substance crystallized out, which after recrystallization from methanol melted at 184–186°, after sublimation at 206–208°, and had the following composition and properties: $C_{30}H_{50}O$ (mass number 426), (Calc.: C, 84·44; H, 11·81; found: C, 84·56; H, 11·79%) $[\alpha]_D^{20}+20°$, $c=3·694$, $CHCl_3$, $\nu_{max}^{CCl_4}$ 887, 1640, 3070 cm^{-1} (exomethylene double bond), 3600 cm^{-1} (OH group). The acetate, m.p. 206°, $C_{32}H_{52}O_2$ (mass number 468), $[\alpha]_D^{20}+38°$, $c=1·152$, $CHCl_3$, $\nu_{max}^{CCl_4}$ 880, 1640, 3070 cm^{-1} (exomethylene), 1732, 1247, 1028 cm^{-1} (acetyl group).

Angelyljaponicin and Kablikopetasin

According to TLC fractions 7–10 contained angelyljaponicin and kablikopetasin. Repeated chromatography on silica gel with light petroleum–ether (the ether content was steadily increased) separated the substances, which were then compared with the substances isolated from *P. kablikianus*. Kablikopetasin, m.p. 78°, $C_{20}H_{28}O_3$ (mass number 316). (Calc. C, 75·91; H, 8·92; found: C, 76·01; H, 8·79%) $[\alpha]_D^{20}-19·28°$, $c=3·214$, $CHCl_3$, $\nu_{max}^{CHCl_3}$ 1700, 1650, 1150, 1240, 1565 cm^{-1}. Angelyljaponicin, $C_{20}H_{28}O_4$; $\nu_{max}^{CHCl_3}$ 1568, 1645, 1700, 3600, 3495 cm^{-1} represented the main part of the fraction. In view of the fact that we have not obtained this substance in a crystalline state as yet, we have characterized it via a crystalline derivative formed by alkaline hydrolysis. Japonicin melted at 196°, $C_{15}H_{22}O_3$ (mass number 250). (Calc. C, 71·97; H, 8·86; found: C, 71·84; H, 8·68%) $[\alpha]_D^{20}-117°$ (CH_3OH; $c=0·4128$).

*Kablicin**

Fractions 11–13 contained almost pure kablicin. A part of it was purified by chromatography on silica gel (elution with benzene + 1% ethanol). A crystalline substance was obtained, m.p. 70–72°, $C_{25}H_{34}O_6$ (mass number 430), $[\alpha]_D^{20}-18·5°$, $\lambda_{max}^{CHCl_3}$ 3590, 1700, 1642, 1562 cm^{-1}, which was characterized by reduction with lithium aluminium hydride in tetrahydrofuran, affording a crystalline triol, m.p. 202°, $[\alpha]_D^{20}-7·0°$ (CH_3OH; $c=0·3943$). (Calc. for $C_{15}H_{28}O_5$: C, 62·47; H, 9·79. Found: C, 62·55; H, 9·70%).

* For the preliminary paper concerning the structure of kablicin see: S. NOVOTNÝ, Z. SAMEK, V. HEROUT, and F. ŠORM, *Tetrahedron Letters* **11**, 1401 (1968).