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SESQUITERPENES FROM *PETASITES**

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Dedicated to Professor Dr. MATTHIAS PAILER, Organisch-Chemisches Institut der Universität Wien, at the occasion of his sixtieth birthday

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Key Word Index—*Petasites*; Compositae; chemotaxonomy; sesquiterpenes; eremophilanes.

Abstract—From the 'inflorescence buds' of *Petasites* hybridus Mill we isolated 2α-angelyloxy-9-oxo-10αH-furoeremophilane (IX), a new eremophilane-type compound. Isopetasin (XIII) was found in *P. kablikianus*, and bakkenolide A (XIV) in both *P. japonicus* subsp. giganteus and *P. albus*. In combination with other results on sesquiterpenoids in plants, these facts have been used to determine the generic relations between *Petasites* species.

INTRODUCTION

WE HAVE studied intensively the sesquiterpenes present in representatives of the genus *Petasites* Mill for more than 10 years. The result of these studies is described in a series of communications on the isolation and the structural determination of substances of the eremophilane type.[1] Later we concentrated our attention on genera in the tribe Senecioneae.[2,3] In view of analyses of components of other genera of this tribe,[4–6] we were able to show that substances of the eremophilane type represent an important chemotaxonomical character in the Senecioneae.[7] By using the state of oxidation of eremophilane derivatives as a character in individual *Petasites* species in combination with morphological information, we were able to determine some intergeneric relations of the taxa[8,9] studied. In earlier papers,[8,9] our attention was concentrated mainly on *P. kablikianus* Tausch. ex Bercht. and *P. paradoxus* (Retz.) Baumg., and, specifically, on the proof of their hybrid origin from the parent species *P. albus* (L.) J. Gaertn. and *P. hybridus* (L.) Gaertn., Mey, et Scherb.

* Part CCX in the series "On Terpenes". For Part CCIX see J. POPLAVSKI, M. HOLUB, Z. SAMEK and V. HEROUT, *Coll. Czech. Chem. Commun.* **36**, 2189 (1971).

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[9] L. NOVOTNÝ, J. TOMAN and V. HEROUT, *Phytochem.* **7**, 1349 (1968).

During the present work we were struck by the evident coincidence of the components of the indigenous *P. albus* (I–V) and the imported *P. japonicus* (Sieb. et Zucc.) Maxim. subsp. giganteus Kitam.[1] The species *P. japonicus* has been intensively investigated by two groups of Japanese authors who described the isolation and the structure of the so-called bakkenolides or fukinolides[10,11] found in shortened flower stems which had not flowered, the so-called 'inflorescence buds'. Somewhat later we isolated substances of the bakkenolide type from an extract of *Homogyne alpina* (L.) Cass,[12] a genus closely related to the *Petasites*. In view of the discrepancy between the components found by us in the rhizomes of *P. japonicus* and those described by the Japanese authors in the buds of the same species, we considered it necessary to determine whether bakkenolides also occur in the buds of the subspecies *P. japonicus* subsp. giganteus and other European species of the *Petasites*. At the same time we wanted to investigate differences between the occurrence of bakkenolides in andro- and gyno-morphic plants (very often incorrectly indicated as 'male' and 'female' types), and also whether there exist differences in the occurrence of these substances during the entire vegetative phase. Samples of inflorescence buds were collected therefore, not only in autumn when the flower buds are already started, but in early spring and immediately before flowering.

The results of these analyses showed that substances of the bakkenolide type are completely absent in the flower buds of *P. hybridus* and *P. kablikianus*. Major components of the rhizome, 9-hydroxyfuroeremophylane (VI) and furanopetasin (VII), were not present in the inflorescence buds, which instead contained their 9-dehydro derivatives VIII and IX. The structure of these substances suggests an oxidation at position 9 and is accompanied by a configurational change from *cis* to *trans* form.

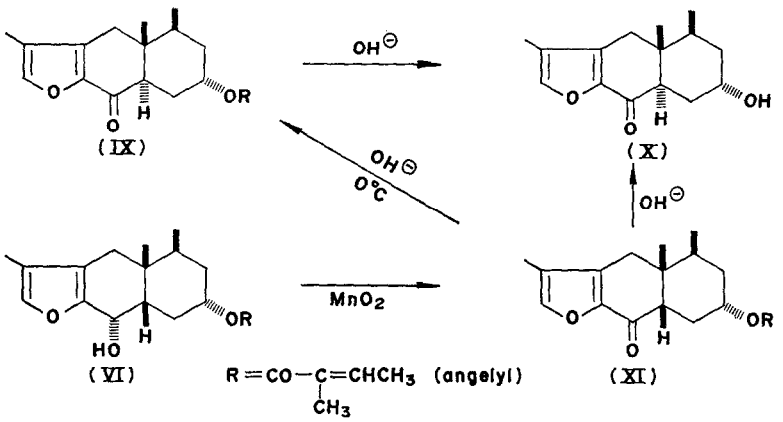
The structure of the previously undescribed derivative IX, ($C_{20}H_{26}O_4$, m.p. 119–120°) was determined from spectral data (see Experimental) and confirmed chemically. Saponification of IX by aqueous-ethanolic sodium hydroxide gave substance X ($C_{15}H_{20}O_3$, m.p. 169–171°) which we isolated from the neutral fraction, and which was shown to be identical with 2α-hydroxy-9-oxo-10αH-furoeremophilane[2] described earlier. In view of the fact that the light petroleum extract of the rhizome of the same taxon contained furanopetasin VI, it was logical to surmise that substance IX is closely connected with furanopetasin. Therefore, we oxidized furanopetasin with manganese dioxide in chloroform and obtained the 9-oxo derivative, XI, with *cis*-fused six-membered rings. This gave, on isomerization with aqueous-ethanolic sodium hydroxide at 0°, substance IX. The course of this reaction is represented in Scheme 1.

The rhizome extracts of *P. kablikianus* contain kablicin (XII) as the main component, while in the buds of the same species kablicin was not found. The main component was in this case isopetasin (XIII), a substance characteristic for rhizomes of *P. hybridus*, chemovar petasin.[8] The finding of this substance in the inflorescence buds of *P. kablikianus* may serve as additional support for the hypothesis of the hybrid origin of the species from the parent taxa *P. hybridus* and *P. albus*—we have already demonstrated.[8]

Bakkenolides were found as the main component of the extracts of the inflorescence buds of the *P. albus* species and of the imported species *P. japonicus* subsp. giganteus. The

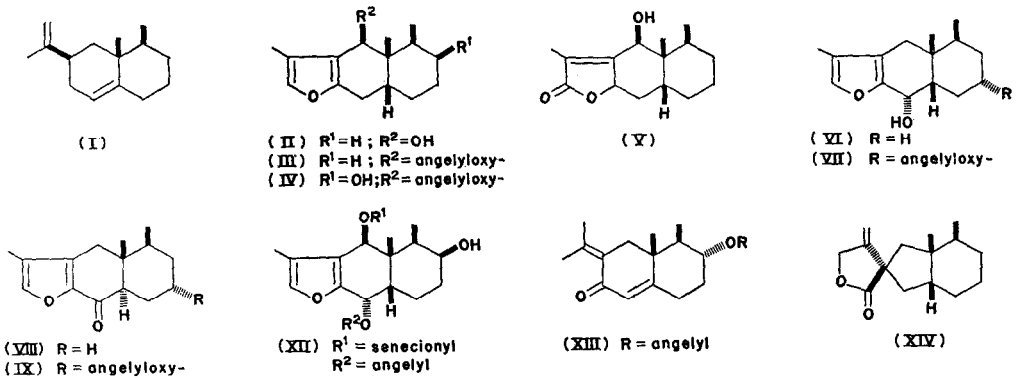
[10] K. SHIRAHATA, T. KATO, Y. KITAHARA and N. ABE, *Tetrahedron* **25**, 3179 (1969).
[11] K. NAYA, I. TAGAKI, M. HAYASHI, S. NAKAMURA, M. KOBASHI and S. KUTSUMURA, *Chem. & Ind.* 318 (1968).
[12] J. HARMATHA, L. NOVOTNÝ, V. HEROUT and F. ŠORM, *5th Intern. Symp. Chem. Natural Products, London* p. 279 (Abstracts) (1968).

major component in the extracts of the buds of both taxa was bakkenolide A (XIV). In view of the fact that the same substances were found in rhizome extracts of both species, we believe that bakkenolides A probably have originated from the same precursor present



SCHEME 1.

in both species, *P. albus* and *P. japonicus*. As the bakkenolides are partly transformed eremophilanes and as the prevailing component in the rhizome extracts of both these species is petasalbin (II), we consider it plausible that the latter compound is a probable precursor of bakkenolide A (XIV).



As the rhizomes of both *Petasites* contain the same components of the eremophilane type and also show an identical occurrence of bakkenolide A in their buds, and since the morphological similarity of their vegetative organs is striking, it can be concluded that the European species *P. albus* is substituted in East Asia by the vicarious species *P. japonicus*. Both species are evidently closely related in spite of their present geographical isolation, while their relation to *P. hybridus* species is evidently more remote. Thus, *P. albus* and *P. hybridus* species can be mutually crossed but preliminary attempts to hybridize *P. albus* with *P. japonicus* subsp. giganteus were negative. This data suggests the following order of the species: *P. hybridus*, *P. japonicus* and *P. albus*; all three species belong to independent sections of the genus. On the other hand, the species *P. kablikianus*, *P. paradoxus* and *P. albus* are members of the same section of the genus, not only on mere morphological

ground, but also by having similar substances, or even, in the case of kablicin by identical substances.[13] We can expect in *P. paradoxus* the occurrence of dehydroderivatives corresponding to the mode of transfer during its hybridization as discussed in previous communication.[8,9]

The results presented here are also interesting from the point of view of biogenetic relations, because in some taxa there is an alternation of main components of various terpenoid types during the vegetative and non-vegetative stage. The study of components present in various organs and those during the whole ontogenic development seems to be promising for the determination of the biogenetic relationships between taxa.

We were unable to find either qualitative or quantitative differences in the components isolated between the materials collected in the autumn or in the spring. No differences could be detected between andro- and gynomorphous specimens.

The frequency of the types of oxidation patterns of substances from light petroleum extracts of the rhizomes of all the species studied is given in Table 1.

TABLE 1. OXIDATION PATTERN OF THE FUROEREMOPHILANE DERIVATIVES IN PETASITES SPECIES

Taxa	Oxidation pattern
P. albus	predominantly 6; seldom 3; often 3 and 6
P. japonicus	uniform occurrence of oxidation at positions: 3; 6; 3 and 6; 3, 6, 9 (effect of *P. hybridus*)
P. kablikianus	
P. paradoxus	
P. hybridus	predominantly 9; seldom 3; often 2 and 9

EXPERIMENTAL

NMR were determined in $CDCl_3$ using Me_4Si silane as internal standard. For TLC Kieselgel G was employed with light petroleum, Et_2O, C_6H_6, EtOH, and mixtures as solvents. Detection was with H_2SO_4 and heating. For column chromatography, silica gel was used (13% H_2O). Alumina of a medium activity (6% H_2O) was also used.

Extraction. Inflorescence buds of *P. albus*, *P. hybridus* and *P. kablikianus* collected in the autumn of 1967 and the spring of 1968 in Malá Úpa in the Giant Mountains (Krkonoše) were used. Buds of *P. japonicus* subsp. giganteus were collected at the same time in the Botanical Garden of the Institute of Botany, Czechoslovak Academy of Sciences, Průhonice near Prague. The buds were frozen, crushed, and extracted with Me_2CO. The extract was concentrated and the residue extracted with benzene.

Chromatography of benzene extract. The dried concentrated benzene extracts were chromatographed on a column of alumina (act. III) and the fractions combined after examination by TLC were further purified by chromatography on silica gel. Individual fractions were examined by IR spectroscopy in $CHCl_3$ and combined on this basis. Only fractions which were evidently of furoeremophilane type (characteristic color with H_2SO_4 and typical IR spectra), or contained an α,β-unsaturated ketone group (petasine types) or an α,β-unsaturated lactone group (eremophilanolides) or were of bakkenolide type were kept.

Isolation of furoeremophilane derivatives from Petasites hybridus *buds.* The benzene extract from 2 kg of fresh buds (30 g) was chromatographed on neutral alumina (act. III; 2 kg). 1 l. fractions were collected. 7 l. of light petroleum, 11 l. benzene, and 19 l. of a benzene–EtOH mixture (4:1) were used for elution.

Furoeremophilone VIII. Rechromatography of fractions 11–13 (1 g) from the preceding chromatography, on silica gel (100 g) with light petroleum–Et_2O (9:1) gave a crystalline fraction which from Et_2O had m.p. and m.m.p. 148–150°. $[\alpha]_D^{24}$ −22·4; UV: λ_{max}^{EtOH} 278 nm (log ϵ 4·10); IR: 1538, 1610, 1696 cm^{-1}. $C_{15}H_{20}O_2$ (232·3) Calc.: C: 77·55, H: 8·68; Found: C: 77·50, H: 8·78%.

2α-Angelyloxy-9-oxo-10αH-furoeremophilane (IX). Repeated chromatography of fractions 11–13 gave a second substance, eluted after VII. Yield 0·2 g, m.p. 119–120°. UV: λ_{max} 217 nm (log ϵ 4·04), 279 nm (log ϵ 4·19). IR: 1240, 1541, 1678, 1700 cm^{-1}. NMR: $H_{(12)}$: 7·36 ppm (bs); $H_{(2)}$: 4·88 ppm (m, ϵ $J \cong 35$ Hz); $H_{(13)}$: 1·99 d ($J_{12,13}$ 1·34 Hz); $H_{(14)}$: 0·98 d (J = 6·5 Hz); $H_{(15)}$: 0·83 s; β-H: 6·04 (angelic acid). $C_{20}H_{26}O_4$ (330·4) calc.: C: 7270, H: 7·93; found: C: 7278, H: 8·00%.

[13] J. TOMAN, *Folia Taxon Geobot.* (in press).

2α-*Hydroxy*-9-*oxo*-10α*H*-*furoeremophilane* (X). Compound IX (80 mg) in EtOH was refluxed with 15 ml 5% NaOH in aq. EtOH for 30 min. The neutral fraction was extracted with Et_2O and the Et_3O dried and evaporated. Crystallization of the residue from Et_2O gave 45 mg m.p. and m.m.p. 169–171°. IR: 1539, 1672, 3600 cm^{-1}. $C_{15}H_{20}O_3$ (248·4) Calc.: C: 72·55, H: 8·12. Found: C: 72·43, H: 8·05%.

Angelic and tiglic acids. The basic fraction from the saponification of compound IX was acidified and extracted with Et_2O. The combined extracts were dried and the residue was methylated (CH_2N_2) and analysed by GLC (2·7 m, 2 mm, Porovina 0·1–0·2 mm, 2% PEG adipate; temp. 100°, N_2 at 19 ml/min); elution times, 2·48 (methyl ester of tiglic acid), 1·56 (methyl ester of angelic acid).

2α-*Angelyloxy*-9-*oxo*-10β*H*-*furoeremophilane* (XI) *and the transformation to substance* IX. To a solution of furanopetasin (1·0 g) in 10 ml $CHCl_3$ 10 g of fresh MnO_2 were added and the suspension shaken for 24 hr. After filtration and washing with $CHCl_3$ the filtrates were evaporated and the residue (which had no furanopetasin, TLC), was dissolved in 5 ml EtOH and added to 5% aq.-alcoholic NaOH at 0°. The solution was quickly evaporated without heating in vacuum and the residue extracted with Et_2O. After washing (H_2O) and drying (Na_2SO_4) Et_2O was evaporated and the residue chromatographed on silica gel. Elution with benzene gave 270 mg of pure IX.

Isolation of substances of furoeremophilane type from petasites albus buds. The benzene extract prepared as above from 4 kg of fresh buds (70 g) was chromatographed on 4·2 kg of neutral alumina, act. III. Elution was as before.

Bakkenolide—A XIV. Rechromatography of fractions 34–36 (3·7 g) from above on silica gel (400 g), using first light petroleum–Et_2O mixture (5:1) gave crystals (2·6 g) which after further purification on a small gel column elution with benzene + 1% EtOH + 1% EtOAc had m.p. and m.m.p. 80–81° UV: λ_{max}^{EtOH} 212 nm (log ε 3·01); IR: 1781, 1670, 3080, 895 cm^{-1}. $C_{15}H_{22}O_2$ Calc.: C: 76·88, H: 9·46. Found: C: 77·00, H: 9·41%.

Isolation of substances of eremophilane type from petasites kablikianus buds. The benzene extract from 3·5 kg fresh buds was chromatographed on neutral alumina, act. III (3250 g) as before.

Isopetasin XIII. From fractions 12–14 of the above chromatography crystals were obtained which from *n*-pentane had m.p. and m.m.p. 99–100°; $[\alpha]_D^{20}$ +31; UV: λ_{max}^{EtOH} IR: 1638, 1678, 1700 cm^{-1}. $C_{20}H_{28}O_3$ Calc. C: 76·08, H: 8·92. Found: C: 76·08, H: 8·90%.

Isopetasol XV. Substance XIII (200 mg) in 5 ml EtOH was added 15 ml of 5% aq.-alcoholic NaOH and refluxed for 30 min. After evaporation of EtOH the neutral fractions were extracted with Et_2O (3 × 50 ml). The Et_2O solution was dried and evaporated. Recrystallization from an Et_2O–EtOAc mixture gave crystals m.p. and m.m.p. 125–126°. The identity was proved by comparison of IR with an authentic specimen.