

COMPONENTS OF *Senecio nemorensis* L. s. str. SUBSP. *nemorensis*

Josef JIZBA, Věra LAUDOVÁ and Ladislav NOVOTNÝ

*Institute of Organic Chemistry and Biochemistry,
Czechoslovak Academy of Sciences, 166 10 Prague 6*

Received May 2nd, 1978

The following components were isolated from *Senecio nemorensis* L. s. str. subsp. *nemorensis* and identified: a mixture of paraffinic hydrocarbons C_{22} — C_{32} , sesquiterpenic hydrocarbons farnesene and bisabolene, β -sitosterol, unsaturated aliphatic alcohols C_{28} — C_{32} , waxes C_{38} — C_{58} , furanoligularone, sesquiterpenic lactones 3-oxo-8 α -eremophila-1,7-dien-8,12-olide, 3-oxo-8 α -hydroxyeremophila-1,7-dien-8,12-olide and an undetermined carotenoid $C_{40}H_{56}O_2$.

Considerable attention has been devoted to the study of the components of the plants of *Senecioneae* tribe, due not only to the content of a number of important alkaloids¹⁻³, but of neutral substances as well. Recently Bohlmann⁴ and his co-workers investigated several tens of species of the *Senecio* genus. In our laboratory several genera of the *Senecioneae* tribe have been studied so far^{5,6}. In this paper our attention is focussed on the components of *Senecio nemorensis* L. s. str. subsp. *nemorensis*. Simultaneously we have also endeavoured to contribute to the evaluation of the taxons of the collective species *S. nemorensis* sp. agg. from the chemotaxonomic point of view. The taxons which we investigated are classified in the literature either in the nominate subspecies *S. nemorensis* L. subsp. *nemorensis*, or they are also considered as an independent subspecies *S. nemorensis* L. subsp. *jacquinianus* (REICHENB.) ČELAK, and as *S. bulgaricus* VELEN. However, the synonymy in the literature⁷⁻⁹ is very often contradictory or unsystematic. The contradiction of the synonymy is often complicated by the evaluation of the taxon *S. fuchsii*, C. C. GMEL, in the literature.

A light petroleum extract of the dry plant (above-ground and underground part) was chromatographed on alumina, and β -sitosterol and a mixture of aliphatic hydrocarbons C_{22} — C_{32} were found and identified in individual fractions. Hydrocarbons with odd numbers of carbon atoms prevail in the mixture, the hydrocarbon C_{29} is the most abundant one. Hydrocarbons with even numbers of carbon atoms were also detected in the mixture, but in comparison with the odd ones they are less abundant. The sesquiterpene hydrocarbons farnesene and bisabolene were also identified in the hydrocarbon fraction; it is not without interest that these hydrocarbons were also found in *Petasites hybridus*^{10,11}. In the more polar fraction a mixture of primary aliphatic saturated alcohols C_{24} — C_{30} was detected. Alcohols

TABLE I
Survey of the Components

<i>S. nemorensis</i>		<i>S. fuchsii</i> ³
Subsp. <i>nemorensis</i>	var. <i>bulgaricus</i> ²	
Paraffinic hydrocarbons C ₂₂ —C ₃₂	paraffinic hydrocarbons C ₂₁ —C ₃₂	^a ^a
Sesquiterpenic hydrocarbons farnesene, bisabolene	—	^a
Primary alcohols with paraffinic chain C ₂₄ —C ₃₂	primary alcohols with aliphatic chain C ₁₇ —C ₃₂	^a
Primary alcohols with olefinic chain C ₂₈ —C ₃₂	—	^a
Waxes C ₃₈ —C ₅₈	waxes C ₃₈ —C ₅₀	^a
β-Sitosterol carotenoid	β-sitosterol —	^a ^a
Furanoligularenone	furanoligularenone	6β-hydroxyeremophil-7(11)-en- -8(12)-enolide
3-Oxo-8α-eremophila- -1,7-dien-8,12-olide	3-oxo-8α-eremophila- -1,7-dien-8,12-olide	nemosenin A (6β-angelyl-oxy- -1β,10β-epoxy-3β-hydroxyeremo- philane)
8α-Hydroxy-3-oxoeremo- -phila-1,7-dien-8,12-olide	8α-hydroxy-3-oxoeremo- -phila-1,7-dien-8,13-olide	nemosenin B (6β-dihydro- -angelyloxy-1β,10β-epoxy- 3β-hydroxyeremophilane)
—	8α-methoxy-3-oxoeremo- -phila-1,7-dien-8,12-olide	nemosenin C (1β,10β-epoxy- -3β-hydroxy-6β-isobutyryloxy furoeremophilane)
—	8α-ethoxy-3-oxoeremophila- -1,7-dien-8,12-olide	nemosenin D (3β-acetoxy- -1β,10β-epoxy-6β-isobutyryloxy- furoeremophilane)
^a	triterpenic alcohols oleanol, lupeol, bauerenol	senemorin (6β-angelyloxy- -1β,10β-epoxyfuroeremophilane)
The valley of the Smolotelský stream near Orlick on Moldau, Czechoslovakia	Vitoša, Bulgaria	Třemešná near Tábor, and Malá Úpa near Trutnov, Czechoslovakia

^a Not worked up.

with an even number of carbon atoms prevail in the mixture. A further group of substances which were identified were primary aliphatic unsaturated alcohols C_{28} — C_{32} , with one double bond. Again alcohols with an even number of carbon atoms predominated over those with an odd number. Another group of substances which was identified are waxes with C_{38} — C_{58} . Palmitic acid is the most important of the aliphatic acids and C_{20} alcohol of the alcohols.

Of the substances of the furoeremophilane type furanologularenone, described earlier by Ourisson and coworkers⁸, was isolated, similarly as in the case of *Senecio nemorensis* var. *bulgaricus*. Furanologularenone crystallized out from the light petroleum extract after partial evaporation of the solvent. In contrast to *S. nemorensis* var. *bulgaricus* the derived oxidation products of furanologularenone were isolated from the light petroleum extract; 3-oxo-8 α -eremophila-1,7-dien-8,12-olide and the more polar 3-oxo-8 α -hydroxy-eremophila-1,7-dien-12-olide. We were unable to isolate any of the 8-alkoxy derivatives present in var. *bulgaricus* from this or from the methanolic extract.

In the methanolic extract after extraction with light petroleum we detected and then isolated from it a still unidentified carotenoid $C_{40}H_{56}O_2$ with two active hydrogens, in addition to the mixtures mentioned above.

As for the chemotaxonomic evaluation of the collective species *S. nemorensis* sp. agg. two species i.e. *S. nemorensis* L. s. str. and *S. fuchsii* C. C. GMEL can be differentiated according to their components. Similarly, *S. nemorensis* s. str. splits into two subspecies, *S. nemorensis* subsp. *bulgaricus* (VELEN.) and *S. nemorensis* subsp. *nemorensis*. The differences in the components of both subspecies are distinctly smaller than between the species *S. nemorensis* and *S. fuchsii*, as evident from Table I.

EXPERIMENTAL

The melting points were measured on a Kofler block. The mass spectra were measured on an AEI-MS-902 instrument. For thin-layer chromatography silica gel G according to Stahl (Merck) was used. Detection was carried out by spraying the plates with conc. sulfuric acid and heating over a direct flame. For column chromatography silica gel was purchased from the firm Hermann (Köln, GFR). Gas chromatographic measurements were carried out on a Perkin-Elmer model F-11 (1) and PYE-104/64 (2) instruments.

Isolation and Identification of Substances from the Rhizomes of *Senecio nemorensis* L. s. str. subsp. *nemorensis*

The whole plant (above-ground and underground parts) was dried at room temperature and the dried material (8.1 kg) extracted with 12 l of light petroleum. The extract (52 g) was chromatographed (chromatography A) on alumina (activity III). Elution with light petroleum (1 l) gave a non-polar fraction (8 g) which was rechromatographed.

Paraffinic hydrocarbons: 8 g of the fraction from chromatography A were rechromatographed on alumina of activity IV–V (chromatography B). The solid residue eluted with 0.6 l of light petroleum in the first fraction of chromatography B was a mixture of paraffinic aliphatic hydrocarbons with C_{25} – C_{35} . They were identified by gas chromatography on chromatograph 2 with an all-glass column 1.5 m long and with 4 mm diameter, packed with Gas-Chrom Q 80–100 mesh with 3% of SE-30, at 250°C. Carrier gas N_2 (50 ml/min), with FID. Paraffinic hydrocarbons with odd numbers of carbon atoms predominate in the mixture over the hydrocarbons with even numbers of carbons.

Farnesene, bisabolene: The first fraction of chromatography B afforded mother liquors (0.9 g) after separation of paraffinic hydrocarbons, which were rechromatographed (chromatography C). Light petroleum (120 ml) eluted a fraction which seemed to be pure. Mass spectrometry indicated M^+ at mass 204. On comparison with standards gas chromatography proved the presence of sesquiterpenic hydrocarbons farnesene and bisabolene. The measurement was carried out on the chromatograph 1 provided with a stainless steel capillary column 50 m long and 0.5 mm I.D. coated with the phase OV-17. Temperature 210°C, carrier gas nitrogen, FID.

Waxes: From the next fraction of chromatography B, eluted with light petroleum–benzene 9 : 1 (1.5–2 l), a substance of m.p. 65–73°C was obtained. Mass spectrometry demonstrated that it consisted of waxes C_{38} – C_{58} . Ester C_{42} predominated in the mixture. Palmitic and then stearic acids were the main ones in the mixture; their ratio was 4 : 1. Of aliphatic alcohols the alcohols with C_{16} – C_{30} predominated, with a maximum at C_{20} .

Aliphatic alcohols: Using more polar solvents in chromatography B a mixture of aliphatic primary alcohols C_{24} – C_{30} was isolated (using benzene–methanol 9 : 1) and their identity demonstrated by mass spectrometry. Alcohols with an even number of carbon atoms predominated in the mixture.

Furanoligularenone: On elution with light petroleum–benzene (1 : 1, 2 l) crude furanoligularenone was isolated, m.p. 78–91°C which after triple crystallization from benzene–acetone afforded a preparation with m.p. 94°C, undepressed on admixture of an authentic preparation.

Unsaturated aliphatic alcohols: From chromatography A a fraction was obtained on elution with light petroleum–benzene (1 : 1) which crystallized out after partial evaporation of the solvent, representing a mixture of aliphatic saturated alcohols. The mother liquors (2.3 g) were chromatographed on 230 g of silica gel deactivated with 13% of water. Elution with benzene–methanol (9 : 2.5) gave a mixture of unsaturated aliphatic alcohols with one double bond, C_{28} – C_{32} in which alcohols with an even number of carbons predominated over those with an odd number.

β -Sitosterol: Elution with benzene–ether (4 : 1) in chromatography A gave a fraction in which β -sitosterol of m.p. 137°C was detected. On mixture melting point determination no depression was observed.

3-Oxo-8 α -eremophilan-8,12-olide: In the subsequent fraction of chromatography A (after β -sitosterol), obtained on elution with benzene–ether (1 : 1) mixture (4 l), a crystalline material was obtained after evaporation of the solvent. The crystals were filtered off under suction and washed on the filter with acetone diluted with light petroleum (1 l). As according to thin-layer chromatography the material obtained (0.5 g) was a mixture of two compounds with about 10% of 8 α -hydroxy derivative, it was chromatographed on a silica gel column (50 g, deactivated with 13% of water), using a benzene–ether mixture (4 : 2.5) for elution. With 350 ml of the mixture a substance was eluted melting at 182–184°C. The melting point was undepressed on admixture of an authentic specimen. In addition to this, the mass spectrum contained the molecular peak at mass 246 and the fragmentation pattern coincided with the spectrum of 3-oxo-8 α -eremophilan-

-1,7-dien-8,12-olide. CD spectrum (dioxane): $\Delta\epsilon_{348} -1.40$; $\Delta\epsilon_{334} -1.69$; $\Delta\epsilon_{280} -0.07$; $\Delta\epsilon_{226} -24.00$.

8 α -Hydroxy-3-oxo-eremophilan-1,7-dien-8,12-olide

Continuing in chromatography A with a solvent with a still further increased polarity (ether-methanol 9 : 1) a fraction was obtained (3.8 g) which was rechromatographed on deactivated silica gel first with benzene and then with benzene-ether (4 : 1; 7 l), affording a material which contained 3-oxo-8 α -hydroxyeremophila-1,7-dien-8,12-olide almost exclusively. After crystallization from benzene-acetone it melted at 208–210°C, undepressed on admixture of an authentic sample. Mass spectrum for C₁₅H₁₈O₄ M⁺ 262:1211, calculated: 262:1205. The agreement with the authentic sample was also confirmed by chromatographic data (thin layer of silica gel G, benzene-acetone 8.5 : 1.5).

Methanolic Extract

After extraction of the whole plant with light petroleum, it was further extracted with methanol. After evaporation of the solvent 150 g of extract were obtained. A column of alumina (act. IV) was then charged with 50 g of the mixture. Using gradient elution not only the same components were isolated as in the chromatography of the light petroleum extract, but also a red-orange substance (with benzene-ether 1 : 1) melting at 175–179°C. Its mass spectrum M⁺ 568, C₄₀H₅₆O₂, with two hydrogen atoms exchangeable for deuterium, indicates that the substance is probably a carotenoid.

For the botanical identification of the plants and for advice concerning the chemotaxonomic part we thank Dr J. Toman. For the measurement of the CD spectra our thanks are due to Dr S. Vašíčková. The mass spectra were measured by Mrs M. Vokáčová and interpreted by Dr L. Dolejš. For gas chromatographic measurements we thank Dr V. Lukeš, Mrs S. Holubová and Mr K. Konečný.

REFERENCES

1. Nguyen Thi Nghia, Sedmera P., Klásek A., Boeva A., Drjanovska L., Dolejš L., Šantavý F.: This Journal 41, 2592 (1976).
2. Klásek A., Mnatsakanyan V. A., Šantavý F.: This Journal 40, 2524 (1975).
3. Leonard N. J. in the book: *The Alkaloids-Chemistry and Physiology* (R. H. F. Manske, Ed.), Vol. VI., p. 35. Academic Press New York—London 1960.
4. Bohlmann F., Knoll K. H., Zdero C., Mahanta P. K., Grenz M., Suwita A., Ehlers D., Le Van N., Abraham W. R., Natu A. A.: *Phytochemistry* 16, 965 (1977).
5. Jizba J., Samek Z., Novotný L., Najdenová E., Boeva A.: This Journal 43, 1113 (1978).
6. Novotný L., Krojídlo M., Samek Z., Kohoutová J., Šorm F.: This Journal 38, 739 (1973).
7. Beger H. in the book: *Illustrierte Flora von Mittel-Europa* (G. Hegi, Ed.). Vol. 6, p. 758. Lehmanns Verlag, München 1928.
8. Chater A. O., Walters S. M. in the book: *Flora Europea* (T. G. Tutin, V. H. Heywood, N. A. Burges, D. M. Moore, D. H. Valentine, S. M., Walters, D. A. Webb, Eds), Vol. 4, p. 196. Cambridge University Press, Cambridge 1976.
9. Ehrendorfer F. (Ed.): *Liste der Gefäßpflanzen Mitteleuropas*, Ed. 2, p. 254. Fischer, Stuttgart 1973.
10. Hochmanová J., Novotný L., Herout V.: This Journal 27, 1870 (1962).
11. Patil F., Ourisson G., Tanahashi Y., Wada M., Takahashi T.: *Bull. Soc. Chim. Fr.* 1968, 1047.

Translated by Ž. Procházka.