COMPONENTS OF *Senecio nemorensis* VAR. *subdecurens**

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From the root of *Senecio nemorensis* L. var. *subdecurens* GIRSB. ex PANTOCS. a mixture of the following compounds was isolated: sesquiterpenic hydrocarbons of mass 204 and paraffinic hydrocarbons C_{11}—C_{13}, a secondary paraffinic alcohol — ginnol a mixture of triterpenic alcohols bauerenol and lupeol, a mixture of caretenoids, a mixture of phytosterols, *i.e.* β-sitosterol, stigmasterol, D-aromadendren-4-ol (spathulenol) (*I*), D-aromadendrane-4β,10α-diol (*III*), and nemosenin E (1β,10β-epoxy-3β-hydroxy-6β-isovaleryloxyfuroeremophilane (*X*).

In the continuation of our study of components of plants from the *Senecioneae* (*Asteraceae*) tribe[1,2] the species *Senecio nemorensis* L. var. *subdecurens* GRISB. ex PANTOCS. was selected. A methanolic extract of the roots was separated to a basic and a neutral fraction. The alkaloidal fraction was investigated by Šantavý and coworkers[3,4]. Chromatography of the neutral fraction on alumina and rechromatography on silica gel afforded the following compounds: In the least polar fraction paraffinic hydrocarbons C_{11}—C_{13} and eight sesquiterpenic hydrocarbons of mass 204 could be detected using the GC/MS technique. In the subsequent fraction the paraffinic secondary alcohol ginnol was isolated, which was described as a component of the fruits and the leaves of *Ginkgo biloba* L. (ref.[5,6]), but in other plant species as well[7,8]. Further the known mixture of triterpenic alcohols bauerenol and lupeol, accompanying the components of *Senecio* genus was isolated and the identity of these alcohols proved by comparison of free alcohols and their acetates in gas chromatography. In further fractions from chromatography on alumina and subsequent chromatography on silica gel a mixture of two carotenoids was isolated, the mass spectra of which indicate the composition $C_{40}H_{64}O_3$ and $C_{40}H_{56}O_3$. In comparison with the content of other substances β-sitosterol was obtained in a relatively large amount, in admixture with campesterol and stigmasterol. Of other sesquiterpenic compounds spathulenol[9,10] (*I*) was isolated, which was recently obtained from *Matricaria chamomila*[11]. The corresponding chromatographic fraction, obtained by chromatography on alumina, was purified by HPLC. D-Aromadendren-4-ol (spathulenol) was identified by comparison with the mass and the NMR spectra from literature (ref.[10]).

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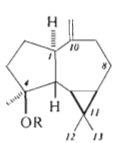
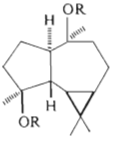
A more polar substance, related with *I*, is its hydroxy derivative *III*. A characteristic feature of both compounds is their coloration with conc. sulfuric acid on thin layers. Red spots are formed at room temperature. The mass spectrum, with M^+ 238·1929, indicates the composition $C_{15}H_{26}O_2$, *i.e.* a molecule containing H_2O more than spathulenol. The frequency 3450 and 3628 cm^{-1} in the IR spectrum indicate the presence of a hydroxy group. The decisive information on the structure of the substance under discussion was obtained from its ^{1}H-NMR spectrum. It showed characteristic signals of two tertiary methyl groups at 1·04 (6 H) and two methyl groups at 1·18 and 1·25, further signals of two cyclopropane protons at 0·65 (1 H, multiplet) and 0·43 (dd, $J_1 = 9{\cdot}5$; $J_2 = 11$). Using *in situ* acylation of the OH group with trichloroacetyl isocyanate (TAI-method[12]) and the characteristic shifts of the tert-methyl signals at 1·18 and 1·25 to 1·50 and 1·60 ppm, these signals were assigned to CH_3—C—OH tert-methyl groups.

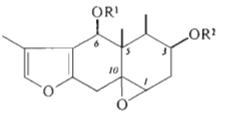
From the comparison of the spectra (Table I) it followed that it is a substance formally derived from spathulenol on addition of a molecule of water on the exo-methylene group at $C_{(10)}$. The configuration of the OH group at $C_{(10)}$ was proposed on the basis of the chemical shift of the signal H_1 at 2·65 ppm in the ^{1}H-NMR spectrum of the trichloroacetylcarbamoyl derivative. The probability of the assignment of this signal of proton H_2 followed from decoupling experiments and the multi-



I, R = H
II, R = CONHCOCCl$_3$



III, R = H
IV, R = CONHCOCCl$_3$



V, $R^1 = R^2 = H$
VI, $R^1 = (Z)$—CH_3CH=C(CH$_3$)—CO, $R^2 = H$
VII, $R^1 = CH_3CH_2CH$(CH$_3$)—CO, $R_2 = H$

VIII, $R^1 = (CH_3)_2CHCO$, $R^2 = H$
IX, $R^1 = (CH_3)_2CHCO$, $R^2 = CH_3CO$
X, $R^1 = (CH_3)_2CHCH_2CO$, $R^2 = H$

plicity observed. In view of the fact that the proton signals in ^{1}H-NMR spectrum of the unacylated compound are localized in the region up to 2 ppm, the appearance of the signal H_1 at 2·65 ppm in the spectrum of the acyl derivative is due to the β-acylation shift of about 0·65 ppm downfield. This shift is in agreement with the proposed position of the OH group on $C_{(10)}$, and by its value it indicates *syn*-configuration of H_1 and $C_{(10)}$—OH (ref.[12]) and hence, under the assumption of α-configuration of H_1, also α-configuration on $C_{(10)}$. Therefore the configuration of D-aromadendrane-4β,10α-diol should be assigned to the substance.

From *S. nemorensis* subs. *fuchsii* the furoeremophilane derivatives nemosenin A (*VI*), nemosenin B (*VII*), nemosenin C (*VIII*) and nemosenin D (*IX*) were isolated and identified[13]. On the basis of their IR spectra and thin-layer chromatographic data it was shown that *S. nemorensis* var. *subdecurens* also contains compounds of furoeremophilane type. A substance was isolated with properties similar to nemosenins, differing, however, from the compounds of this type described so far, by the esterifying acid. It was given the name nemosenin E (*X*). Its mass spectrum indicated M^+ 348 and contained a further characteristic peak at m/z 246 (M − 102), corresponding to alcohol *V*. The spectrum also contained a characteristic peak at m/z 103, corresponding to the ions of protonated acid. From these ions water and carbon monoxide are gradually eliminated as neutral fragments. In addition to this a neutral fragment, C_3H_7, is also split off from the ion of the protonated acid. On the basis

TABLE I
Comparison of the Characteristic Signals in the ^{1}H-NMR Spectra

Compound	H_6	H_7	H_{12}, H_{13}	H_{14}	H_{15}
I[a]	0·45 dd (9·5; 11·5)	—	1·03; 1·05	1·26	4·68 (2 H)
V[a,b]	0·52 dd	—	1·05 (6 H)	1·64	4·7 (2 H)
III	0·43 dd	0·65 m	1·04 (6 H)	1·26	1·18
IV[b,c,d]	0·52 dd (9·5; 11)	0·72 m	1·05 (6 H)	1·60[e]	1·50[e]

[a] Measured on a Varian HA—100 (100 MHz) instrument; solvent deuteriochloroform; tetramethylsilane as internal reference, chemical shifts in δ-scale, splittings in Hz; [b] prepared by in situ acylation with trichloroisocyanate (CCl_3CONCO); TAI-method[12]; [c] measured on a Varian XL—200 instrument (200 MHz); further see note a; [d] proton H_1: 2·65 q (9·5); [e] tentative assignment on the basis of the analogy of the signals of H_{14} in *I* and *II*.

of the intensity ratio of the peaks of ions m/z 43 and 57 and the elimination of the fragment C_3H_7 it may be deduced that isovaleric acid residue must be involved. In agreement with this the ^{1}H-NMR spectrum also contained a doublet of two secondary methyl groups at 0·98 ppm (6 H; J = 6·8). A confirmation of the structure of the alcoholic component was the saponification of the fraction which contained nemosenin E. After hydrolysis with aqueous–ethanolic alkali 1β,10β-epoxy-3β,6β-dihydroxyfuroeremophilane (*V*) was isolated. In the acid fraction after the saponification a mixture of fatty acids was isolated. After their esterification with diazomethane the presence of an ester of a C_6 branched fatty acid was confirmed by the GC/MS method, containing one double bond in the form of *Z*- and *E*-isomers. It is probable that this acid is a component of a further nemosenin.

Among the isolated and identified compounds the presence of furoeremophilane derivative *X* is characteristic of *Senecio nemorensis* var. *subdecurens*.

EXPERIMENTAL

The melting points were determined on a Kofler block. The IR spectra were measured on a UR-20 instrument and the mass spectra on an AEI-MS 902 spectrometer. The ^{1}H-NMR spectra were measured on Varian HA—100 and XL—200 instruments. For thin-layer chromatography silica gel G according to Stahl (Meck) was employed, detection was carried out by spraying with conc. H_2SO_4 and heating with a direct flame. Column chromatographies were carried out on alumina Reanal (Hungary) and silica gel from the firm Hermann (Köln, FRG). Gas chromatographic measurements were carried out with a Pye—104 (model 64) chromatograph. The isolation of some substances was carried out on a non-commercial HPLC.

Isolation and Identification of Compounds from the Methanolic Extract

The roots of *Senecio nemorensis* L. var. *subdecurens* GRISB. ex PANTOCS (15 kg) were collected in the Bulgarian mountains Vitoša and dried at room temperature. After grinding they were extracted 7 times with 30 l of methanol. After evaporation and concentration under reduced pressure to 2 l volume the extract was diluted with water (1 : 1) acidified with a solution of citric acid and extracted twice with a mixture of light petroleum and ether (1 : 1) and three times with 400 ml of ether. The combined extracts were washed with a dilute solution of citric acid, water and 10% soda solution. The ethereal layer was dried over sodium sulfate and evaporated. The residue (93 mg) represents the neutral fraction.* Chromatography on alumina (2·5 kg, act. II—III) was carried out with 87 g of the neutral extract. Elution with light petroleum (6 l) gave a fraction of hydrocarbons.

Mixture of sesquiterpenic and paraffinic hydrocarbons: In this fraction hydrocarbons were detected using the GC/MS method on a tandem of Pye ser. 104 model 64 chromatograph and an AEI MS 902 mass spectrometer, using a separator according to Watson and Biemann. The mixture was chromatographed on a 4 mm column, 150 cm long, packed with 3% SE 30 on Gas

* We thank Professor F. Šantavý, Palacký University, Olomouc, and Dr A. Klásek, Department of Organic Chemistry, Tannery Institute, Gottwaldov, for kindly supplying the raw material.

Chrom Q (100—120 mesh). The starting temperature of the chromatographic column was 40°C and it was kept at this temperature for 10 min and then increased at a 2°C/min rate up to 200°C. The temperature of the ion source was 150°C and the energy of the electrons 70 eV. The mixture contains eight sesquiterpenic hydrocarbons of mol. mass 204 and a mixture of paraffinic hydrocarbons: undecane, dodecane and tridecane.

Ginnol: Using a mixture of light petroleum and benzene (90 : 10) and a light petroleum–benzene mixture (1 : 1) (7 l) a fraction was obtained from which a compound crystallized out which was relatively pure and when crystallized from acetone a preparation, m.p. 83—83·5°C, which gave in mass spectrometry the molecular peak M^+ at 424, further m/z 406 (M—H_2O). A mixture with authentic ginnol had an undepressed melting point.

Mixture of carotenoids: Using a mixture of light petroleum and benzene (1 : 1, 3 l) and benzene (4 l) for elution a further fraction was obtained which was red-coloured. The volatile components were partly distilled off and the non-volatile part, containing carotenoids, was eluted with pentane from the crystallizing residue. After evaporation of pentane it was chromatographed on silica gel (100 g, desactivated with 13% of water) with a mixture of light petroleum and acetone (0·1—0·5% of acetone) to yield a fraction which contained two carotenoids. M^+ 608·4781 indicates the composition $C_{40}H_{64}O_4$ (calculated value 608·5804), for M^+_2 584·4240 the composition was $C_{40}H_{56}O_3$ (calculated 584·429). UV spectrum λ_{max} 408 nm for the mixture of both carotenoids.

Mixture of bauerenol and lupeol: In the solid fraction freed of carotenoids with pentane the triterpenes bauerenol[1] and lupeol were detected and identified by gas chromatography on 3% SE—30 at 270°C, both as free alcohols and acetates.

D-*Aromadendren*-4-*ol* (*spathulenol*) (I): During the isolation of carotenoids and triterpenic alcohols the volatile fraction was distilled off in a vacuum at 0·2. Torr and 185°C bath temperature. On thin-layer chromatography in light petroleum–acetone 9 : 1 a spot could be detected which after spraying with sulfuric acid rapidly turns red. Using preparative thin-layer chromatography with light petroleum–acetone 9 : 1 the substance could be obtained in about 90% purity. On repeated thin-layer separations the sesquiterpenic alcohol could be obtained pure. IR spectrum: 3450, 3628 cm^{-1} (hydroxy group bands), 888, 1640, 3075 cm^{-1} (exomethylene group). Mass spectrum: M^+ 220, further peaks m/z 205, 202, 187, 177, 162, 159, 119. $[\alpha]_D$ +33·7° (c 0·03 chloroform).

Mixture of phytosterols: Elution with benzene (4 l) and benzene–ether mixture (9 : 1, 3 l) a fraction was obtained which afforded a considerable amount of a mixture of phytosterols. Their identity was determined by mass spectrometry.

Nemosenin E (1β,10β-epoxy-3β-hydroxy-6β-isovalerylfuroeremophilane, *X*)

Applying chromatography on alumina (chromatography A) with benzene–ether (from 10—15% of ether, 13 l) a fraction was obtained which in the IR spectrum contained bands at 1720 and 3540 cm^{-1}. The fraction was rechromatographed on silica gel, using benzene–ether (2—5% of ether, 3 l). The eluted fractions containing the required substance were rechromatographed under the same conditions, affording a relatively pure substance. Mass spectrum: M^+ 348, m/z 246 (M—C_5 acid), 103 (protonated C_5 acid), 85 (103—H_2O), 57 (base peak), 75 (103—CO, m*), further peaks at m/z 43, 173, 188, 199, 231. — ^{1}H-NMR spectrum ($CDCl_3$, 60 Mc); 3·10 (H_1); 4·23 (H_3); 6·36 (H_6); 7·06 (H_{12}); 1·80 (H_{13}); 1·20 (H_{15}).

A part of the fraction was saponified with 5% aqueous-ethanolic NaOH (1 h refluxing). From the alkaline solution the soluble fractions were extracted with ether. Chromatography on alu-

mina (Woelm) with light petroleum–ether 1 : 1 gave a substance with m.p. 253—256°C (methanol, ethanol), poorly soluble in all common solvents; in thin-layer chromatography even a weak overloading causes tailing. Mass spectrometry gave M^+ 264·1361, *i.e.* $C_{15}H_{20}O_4$ (calculated 264·1361); *m/z* 123 (b.p.); *m/z* 124. On labelling with C_2H_5OD two D enter the ion 123 and one D the ion 124.

The alkaline solution after hydrolysis of the esters was acidified and extracted with ether. The ether extract was esterified with diazomethane. Using the GC/MS method an unsaturated branched aliphatic acid C_6 also could be detected. The measurement was carried out with instruments described in the separation of hydrocarbons. The mixture of esters was chromatographed on a 0·4 × 150 cm column, packed with 3% SE—30 on Gas-Chrom Q (100—120 mesh), using a programmed temperature increase of 3°C/min from 70°C. It was found that the methyl esters of *Z*- and *E*-4-methylpentenoic acid represent some 80% of the mixture. M^+ 128, (M—15), (M—31), (M—32), *m/z* 69 (M—31—28), *m/z* 69 $\xrightarrow[-28]{m^*}$ *m/z* 41.

D-*Aromadendrane*-4β,10α-*diol* (III). From chromatography A a fraction was obtained on elution with ether (5 l) and ether–methanol mixture (5—10% of methanol, 4 l) which was rechromatographed on silica gel deactivated with 13% of water and washed with a mixture of light petroleum and ether (50—60% of ether, 3·5 l). The compound isolated was purified by HPLC (heptane–acetone 25%, 20 atm, 17 ml/min). M.p. 123—124°C. Mass spectrum: M^+ 238·1929, for $C_{15}H_{26}O_2$ calculated: 238·1933; *m/z* 207 and 59. IR spectrum: 3450 and 3628 cm^{-1}.

The IR *spectra were measured by Mr P. Formánek and interpreted by Dr S. Vašičková. The mass spectra were measured by Mrs L. Tipková and a part of the spectra was interpreted by Dr L. Dolejš. Gas chromatography was carried out by Mr K. Konečný and the* HPLC *preparation by Dr T. Vaněk.*

REFERENCES

1. Jizba J., Samek Z., Novotný L., Najdenová E., Boeva A.: This Journal *43*, 1113 (1978).
2. Jizba J., Laudová V., Novotný L.: This Journal *344*, 952 (1979).
3. Klásek A., Sedmera P., Boeva A., Šantavý F.: This Journal *38*, 2504 (1973).
4. Klásek A., Sedmera P., Vokoun J., Boeva A., Dvořáčková S., Šantavý F.: This Journal *45*, 548 (1980).
5. Kawamura J.: Japan. J. Chem. *3*, 89 (1928); Chem. Zentralbl. *II*, 2255 (1928).
6. Furukawa S.: Bul. Inst. Physical Chem. Res. Tokyo *2*, 2 (1929); Chem. Zentralbl. *I*, 1472 (1929).
7. Jizba J., Herout V.: This Journal *39*, 3756 (1974).
8. Matsuo A., Nakayama M., Ishida Y., Hayashi S.: Z. Naturforsch. *26b*, 32 (1971).
9. Bowyer R. C., Jefferies P. R.: Chem. Ind. (London) *1963*, 1245.
10. Juell S. M. K., Hansen R., Jork H.: Arch. Pharm. (Weinheim) *309*, 458 (1976).
11. Motl O., Repčák M., Sedmera P.: Arch. Pharm. (Weinheim) *311*, 75 (1978).
12. Samek Z., Buděšínský M.: This Journal *44*, 558 (1979).
13. Novotný L., Krojidlo M., Samek Z., Kohoutová J., Šorm F.: This Journal *38*, 739 (1973).

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