COMPONENTS OF *Senecio pancicii* DEGEN VAR. *arnautorum* (VELEN.) STOJ., STEF. et KIT. AND *S. Pancicii* DEGEN VAR. *pancicii**

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Dedicated to Professor Dr V. Herout, corresponding member of the Czechoslovak Academy of Sciences, on the occasion of his 60th birthday.

In *Senecio pancicii* var. *arnautorum* and var. *pancicii* a mixture was found of paraffinic hydrocarbons C_{25}—C_{33}, paraffinic alcohols C_{24}—C_{30}, phytosterols, palmitic acid, esters of fatty acids C_{12}, C_{14} and C_{16} with dihydroxytriterpenes (among which one was identified as faradiol) and senecio alkaloids senecionine and seneciophylline.

Further taxons of the *Senecio* genus[1-4] studied by us are *S. pancicii* var. *arnautorum* and *S. pancicii* var. *pancicii*. Complete plants were collected during the flowering period in the Vitosha mountains in Bulgaria. The light petroleum extract containing non-basic compounds was chromatographed on alumina. In the least polar fraction a mixture of paraffinic hydrocarbons C_{25}—C_{33} was found in which hydrocarbons with an odd number of carbon atoms predominate. From the more polar fractions a mixture of paraffinic alcohols was isolated, with straight chains and carbon atom number C_{24}—C_{30}. Unlike the paraffins, in this fraction alcohols with an even number of carbon atoms predominate. The alcohol C_{26} is the main component. Palmitic acid was identified by mass spectroscopy. In addition to this free acid, lauric, myristic and palmitic acid were also detected in more polar fractions, but in bound form, as esters with dihydroxyterpenes. Their fragmentation in the mass spectra indicates that the acids are bound to the hydroxyl in the position 3 of the ring A of the triterpenes. A mixture of these esters was hydrolysed, the acids were isolated and then converted with diazomethane to methyl esters which were identified by GLC

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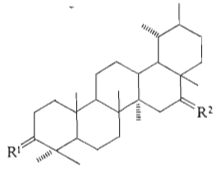
on comparison with authentic samples. From the mixture of dihydroxytriterpenes the triterpenic alcohol faradiol could be isolated by chromatography on silica gel and HPLC. Acetylation of the isolated faradiol gave amorphous diacetate *II*, and oxidation of faradiol with chromium trioxide afforded diketone *III*. Faradiol (*I*) and its derivatives *II* and *III* were described by Pyrek and Baranowska[5]. In subsequent chromatographic fractions a mixture of triterpenic alcohols of the bauerenol group could be identified, in which lupeol predominated. The usual mixture of phytosterols (β-sitosterol, stigmasterol and campesterol) accompanied the other components in relatively large amounts.

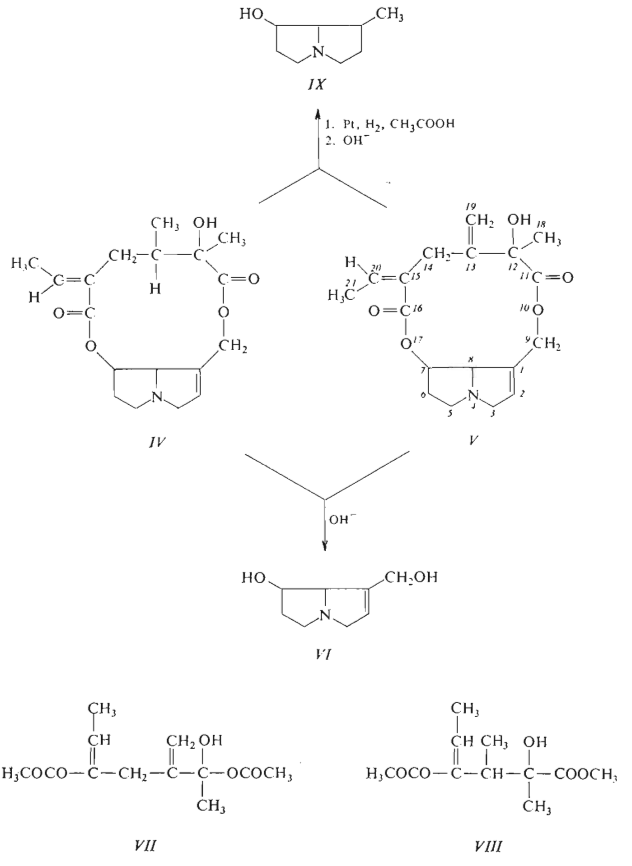


I; $R^1 = \alpha$-H; β-OH, $R^2 = \alpha$-H; β-OH
II; $R^1 = \alpha$-H; β-$OCOCH_3$, $R^2 = \alpha$-H; β-$OCOCH_3$
III; $R^1 = R^2 = O$

The methanolic extract was chromatographed on alumina. From the very polar fraction, containing alkaloids, a mixture of compounds was obtained, melting at 218–220°C, the mass spectrum of which indicated that it is a mixture of two alkaloids with *m/z* 333 ($C_{18}H_{23}NO_5$) and *m/z* 335 ($C_{18}H_{25}NO_5$). The IR, ^{1}H- and ^{13}C NMR spectra and the chromatographic behaviour on thin layers of silica gel[6,7] indicated that it was a mixture of senecionine (*IV*) and seneciophylline (*V*) in a 2 : 3 ratio. Chromatography of the mixture on silica gel impregnated with silver nitrate and crystallization gave pure seneciophylline. A number of authors studied senecionine, seneciophylline and their mixture[7–10] by infrared spectroscopy. Since we could not obtain senecionine in a pure state, the mixture of both alkaloids was hydrolysed. From the alkaline fraction of the hydrolysate retronecine (*VI*) was obtained as the sole product, from which its hydrochloride was prepared, with m.p. 159–161°C (in accordance with ref.[11]). From the acid fraction of the hydrolysate a mixture of dicarboxylic acids was obtained which was converted to methyl esters with diazomethane and separated on silica gel to two substances. The first was identified as dimethyl seneciophyllinoate (*VII*) and the second as dimethyl senecioate (*VIII*). Hydrogenation of the mixture of alkaloids on platinum in acetic acid and subsequent hydrolysis of the hydrogenation product gave retronecanol (*IX*). A study of both taxons indicated that their components are identical.

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 was used. Detection was carried out by spraying the plates with concentrated sulfuric acid and carbonization with direct flame. For column chromatography silica gel of the firm Hermann-Köln,



GFR, was used. Gas chromatographic measurements were carried out on a PYE-104/63 chromatograph. The ^{1}H NMR spectra of triterpenes *I*—*III* were measured on a Varian XL-200 (200 MHz) spectrometer. The ^{1}H and ^{13}C NMR spectra of the mixture of alkaloids *IV* and *V* were measured on a Jeol FX-60 (60 MHz for ^{1}H and 15·03 MHz for ^{13}C) spectrometer. All the NMR spectra were measured using the FT mode and for the measurement solutions of substances in deuteriochloroform were used, with tetramethylsilane as internal reference. HPLC was carried out on an apparatus composed of the following modules: a VCM-300 pump (Developmental workshops of the Czechoslovak Academy of Sciences), Separon Si and C_{18} columns (Laboratory Apparatus, Prague), a Knauer refractometer 2 050, and a TZ 21 S recorder (Laboratory Apparatus, Prague).

Processing of the Plants

Whole plants of *S. pancicii* var. *arnautorum* were dried at room temperature and the dry material (0·60 kg) was ground and extracted. With light petroleum (3 l) 20 g of an extract were obtained, while methanol (6 l) extracted 55 g of material. The variety *pancicii* was worked up in a similar manner, affording 18 g of a light petroleum extract and 50 g of the methanolic extract. Further procedure was similar. The working up of the extracts is described for *S. pancicii* var. *arnautorum*.

Isolation and Identification of the Components

The light petroleum extract (20 g) was chromatographed on alumina (act. II—IV).

Paraffinic hydrocarbons: Elution with light petroleum gave a fraction from which a mixture of hydrocarbons was isolated by gas chromatography on a chromatograph with an all-glass column 1·5 m long, 4 mm I.D., packed with Gas-Chrom Q (80—100 mesh) with 3% SE-30, at 250°C. Carrier gas N_2 (50 ml/min), with FID. In the hydrocarbon mixture (C_{25}—C_{33}) odd hydrocarbons predominate over even hydrocarbons, while the hydrocarbon C_{29} is dominant.

Paraffinic alcohols: In the fraction eluted with light petroleum–benzene mixture (9 : 1) a mixture of paraffinic alcohols C_{24}—C_{30} was present, which were identified on gas chromatograph under the conditions mentioned in the preceding section on paraffins. The paraffinic alcohols C_{24}—C_{30} were present. Alcohols with an even number of carbon atoms predominate. Most abundant was the alcohol C_{26}. In this mixture of alcohols palmitic acid was also present, as shown by mass spectrometry.

Triterpenic alcohols: Elution with a mixture of light petroleum and benzene (1 : 1) gave a mixture of triterpenic alcohols which accompany the components of the *Senecio* genus. This time the mixture contained only bauerenol. These alcohols were determined by gas chromatography under the conditions described for hydrocarbons, with 3% SE-20 at 270°C.

Phytosterols: Elution with a benzene–ether mixture (9 : 1) gave a mixture of phytosterols which was identified by mass spectrometry as a mixture of β-sitosterol, campesterol and stigmasterol.

Esters of fatty acids with dihydroxytriterpenes: Elution with light petroleum–benzene mixture (4 : 1) and then with benzene gave a fraction which could not be separated even after fourfold chromatography. According to gas chromatography the mixture contained three components. According to IR and mass spectra they are esters of higher fatty acids. They were hydrolysed by boiling in 5% ethanolic potassium hydroxide for 6 h. After evaporation of the alcohol the mixture was diluted with water and extracted with ether (*I*). The residue after extraction with

ether was acidified with dilute sulfuric acid and again extracted with ether. Diazomethane was added to the extract and the methyl esters formed were identified by gas chromatography as methyl laurate, myristate and palmitate on comparison with a known mixture of fatty acid methyl esters. The ethereal solution of *I* from alkaline hydrolysis of esters was evaporated and the distillation residue was worked up by means of HPLC on a column of Separon Si 5 μm (6 × 250 mm) in hexane–ethyl acetate (65 : 35), 1·5 ml/min flow-rate. The obtained faradiol (*I*) displayed in its mass spectrum peaks of *m/z* 207 and 189 in addition to the molecular peak 442. ^{1}H NMR spectrum: 0·73 s, 3 H (CH_3); 0·77 s, 3 H (CH_3); 0·86 s, 3 H (CH_3); 0·98 s, 3 H (CH_3); 1·00 d, J = 6·5, 3 H ($C_{(19)}$—CH_3); 1·01 s, 3 H (CH_3); 1·06 s, 3 H (CH_3); 1·66 bs, 3 H ($C_{(20)}$—CH_3);3·21d d, J = 10 2; 5·8, 1 H ($C_{(3)}$—H); 3·44 dd, J = 11·4; 4·8, 1 H ($C_{(16)}$—H); 5·32 bd, J = 6·4, 1 H ($C_{(21)}$—H).

Diacetate II: It was prepared from *I* with acetic anhydride in pyridine. Its mass spectrum showed the following fragmentation: M 526, *m/z* 466 and 406 (small peak). ^{1}H NMR spectrum: 0·80 s, 3 H (CH_3); 0·84 s, 3 H (CH_3); 0·86 s, 3 H, (CH_3); 0·88 s, 3 H (CH_3); 1·00 d, J = 6·5, 3 H ($C_{(19)}$—CH_3); 1·05 s, 3 H (CH_3); 1·06 s, 3 H (CH_3); 1·64 bs, 3 H ($C_{(20)}$—CH_3); 2·02 s, 3 H ($OCOCH_3$); 2·04 s, 3 H ($OCOCH_3$); 4·48 dd, J = 10; 6, 1 H ($C_{(3)}$—H); 4·75 dd, J = 11·5; 4·8, 1 H ($C_{(16)}$—H); 5·25 bd, J = 6·4, 1 H ($C_{(21)}$—H).

Dione III: 20 mg of faradiol (*I*) were oxidized with chromium trioxide in pyridine. The oxidation product was separated by preparative thin-layer chromatography. After isolation it was crystallized from a mixture of acetone and light petroleum, m.p. 240—242°C. Mass spectrum contained M^+ 438 and *m/z* 205 and 150. ^{1}H NMR spectrum: 0·91 s, 3 H (CH_3); 0·97 s, 3 H (CH_3); 1·04 s, 3 H (CH_3); 1·04 d, J = 6·5, 3 H ($C_{(19)}$—CH_3); 1·06 s, 3 H (CH_3); 1·09 s, 3 H (CH_3); 1·16 s, 3 H (CH_3); 1·66 bs, 3 H ($C_{(20)}$—CH_3); 2·48 m, 2 H ($C_{(2)}$—H_2); 1·90 d, J = 13·5, 1 H and 2·83 bd, J = 13·5, 1 H ($C_{(15)}$—H_2); 5·33 bd, J = 6·5, 1 H ($C_{(21)}$—H).

Senecionine (*IV*) and Seneciophylline (*V*)

The methanolic extract obtained after extraction with light petroleum, weighing 50 g, was chromatographed on alumina (act. III). After elution with 5 l of ether and 2 l of a mixture of ether and chloroform (9 : 1) a mixture of alkaloids was obtained (2·3 g) which was rechromatographed. However, a mixture was obtained again the mass spectrum of which indicated the presence of two alkaloids of molecular masses 333 and 335 in a 2 : 1 ratio. The compounds differed by one double bond. Hydrogenation on platinum in acetic acid indicates 3·62 double bonds. Elemental analysis also indicates a mixture of alkaloids of the composition $C_{18}H_{23}NO_5$ (333) and $C_{18}H_{25}$. NO_5 (335) (found 64·60% C, 7·22% H, 4·17% N). IR spectrum: 3 525, 1 718, 1 658, 1 253 cm^{-1}. It signalizes the presence of a CH group and an unsaturated ester group. ^{1}H NMR spectrum: 1·10 d, J = 6·3, 3 H ($C_{(12)}$—CH_3) in *IV*; 5·10 bs, 1 H and 5·30 bs, 1 H ($C_{(12)}$—CH_2) in *V*. ^{13}C NMR spectrum: the assignment was carried out on the basis of the spectrum of compound[12] *IV*; signals common to compounds *IV* and *V*: 15·2 ($C_{(21)}$), 24·8 ($C_{(18)}$), 35·0 ($C_{(5)}$), 38·5 ($C_{(14)}$), 53·3 ($C_{(5)}$), 61·2 ($C_{(3)}$), 62·9 ($C_{(9)}$), 75·0 ($C_{(8)}$), 77·7 ($C_{(7)}$), 131·5 ($C_{(1)}$), 134·2 ($C_{(20)}$), 136·7 ($C_{(2)}$); further signals of compound *IV*: 11·2 ($C_{(19)}$), 37·4 ($C_{(13)}$), 76·3 ($C_{(12)}$), 133·2 ($C_{(15)}$), 167·8 ($C_{(16)}$), 177·0 ($C_{(11)}$); further signals of compound *V*: 77·1 ($C_{(12)}$), 114·5 ($C_{(19)}$), 146·3 ($C_{(13)}$), 136·1 ($C_{(15)}$), 167·1 ($C_{(16)}$), 178·1 ($C_{(11)}$).

Repeated chromatography on silica gel enriched the mixture of both alkaloids on seneciophylline. Chromatography on alumina (Woelm) impregnated with 13% silver nitrate gave a product, which was seneciophylline with about 5—10% of senecionine, m.p. 215—217°C, which on crystallization afforded pure seneciophylline with m.p. 217°C.

Hydrolysis of the mixture of alkaloids: 260 mg of the mixture was refluxed with sodium hydroxide in ethanol. After 6 h the mixture was extracted with hot chloroform. The extract contained retronecine from which its hydrochloride was prepared with ethanolic hydrogen chloride. After crystallization its m.p. was 159–161°C. After acidification of the reaction mixture with dilute sulfuric acid and extraction with ether the extract contained a mixture of senecioic and seneciophyllinoic acids, which were converted to their methyl esters with diazomethane. On chromatography of the mixture on silica gel with light petroleum–acetone mixture (with 3% of acetone) methyl seneciophyllinoate was obtained. Mass spectrum: M^+ 242, *m/z* 225, 224, 211, 210, 197, 193, 183, 151, 139, 123, 109, 59, 43. IR spectrum: 1 725, 1 648, 3 540 cm^{-1}. Further elution afforded methyl senecioate: Mass spectrum: M^+ 244, *m/z* 213, 212, 197, 195, 194, 185, 153, 141, 109, 81, 59, 43.

From the hydrogenation of the mixture of alkaloids in acetic acid (on PtO_2) retronecanol was isolated the mass spectrum of which contained the peaks of M^+ 141, and *m/z* 97, 82 and 69.

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REFERENCES

1. Novotný L., Krojidlo M., Samek Z., Kohoutová J., Šorm F.: This Journal *38*, 739 (1973).
2. Jizba J., Samek Z., Novotný L., Najdenová E., Boeva A.: This Journal *43*, 1113 (1978).
3. Jizba J., Laudová V., Novotný L.: This Journal *44*, 953 (1979).
4. Jizba J., Laudová V., Samek Z., Ubik K., Novotný L.: This Journal, *46*, 1048 (1981).
5. Pyrek J. S., Baranowska F.: Tetrahedron Lett. *1973*, 809.
6. Šantavý F. in the book: *Thin-Layer Chromatography* (E. Stahl, Ed.), p. 532. Springer, Berlin 1969.
7. Bull L. B., Culvenor C. C. J., Dick A. T.: *The Pyrrolizidine Alkaloids*. p. 39, 46 and 280. North-Holland, Amsterdam 1968.
8. Adams R., Govindachari T. R.: J. Amer. Chem. Soc. *71*, 1180 (1953).
9. Culvenor C. C. J., Smith L. W.: Aust. J. Chem. *8*, 556 (1955).
10. Adams R., Gianturco M.: J. Amer. Chem. Soc. *78*, 398 (1956).
11. Adams R., Rogers E. F.: J. Amer. Chem. Soc. *61*, 2815 (1939).
12. Wiedenfeld H., Röder E.: Phytochemistry *18*, 1083 (1979).

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