

# Phytochemical study of *Salvia officinalis* L.

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$\beta$ -Ursolic and  $\beta$ -oleanolic acids, 7-ethylrosmanol, cirsimaritin, luteolin-7-*O*-glucoside, apigenin-7-*O*-glucoside,  $\beta$ -sitosterol, and saccharose were found in the leaves of salvia (*Salvia officinalis* L.). Cirsimaritin and apigenin-7-*O*-glucoside were isolated from this plant species for the first time and the presence of 7-ethylrosmanol in the genus *Salvia* has not been reported as yet.

В листьях пеларгонии (*Salvia officinalis* L.) обнаружены  $\beta$ -урзоловая кислота,  $\beta$ -карофиллин, 7-этилрозманол, цирсимаритин, лутеолин-7-*O*-глюкозид, апигенин-7-*O*-глюкозид,  $\beta$ -ситостерол и сахароза. Цирсимаритин и апигенин-7-*O*-глюкозид были впервые выделены из растений этого вида и о нахождении 7-этилрозманола у растений рода *Salvia* также до сих пор не сообщалось.

Several organic compounds of various biological activity have hitherto been isolated from plants belonging to genus *Salvia* L. as *e. g.* pentacyclic triterpenes [1—3], diterpenes [4, 5], diterpenic quinones denoted royleanones [6, 7], *o*-naphthoquinone diterpenes denoted tanshinones [8], sesquiterpenes [9], flavonoids [10, 11], glycosides [12], as well as essential oil, the amount and composition of which depends on the locality, climatic conditions and the growing stage [13].

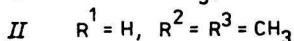
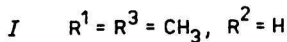
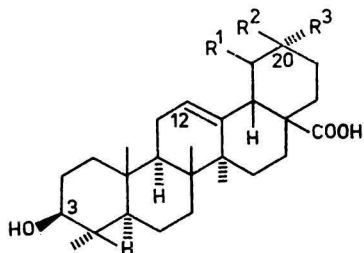
Genus *Salvia* L. implies about 550 species resembling each other; they are under an intense botanic investigation. The above-mentioned compound classes were isolated and identified from more than 50 species of salvia.

Although leaves of *Salvia officinalis* are entered in the Czechoslovak Pharmacopoeia and the curative effect of extracts prepared from them is widely known and exploited, the phytochemical study of components present in *Salvia officinalis* growing in Czechoslovakia has not been effected. This paper concerns the identification of compounds isolated from the aerial part of the plant grown in the Experimental Garden of Medicinal Plants of the Comenius University in Bratislava.

The fourth edition of the Czechoslovak Pharmacopoeia requires under the entry *Herba Salviae* at least a 1 % content of the essential oil. Employment of the given procedure showed the content of essential oil to be 2.1 % (average of five estimations).

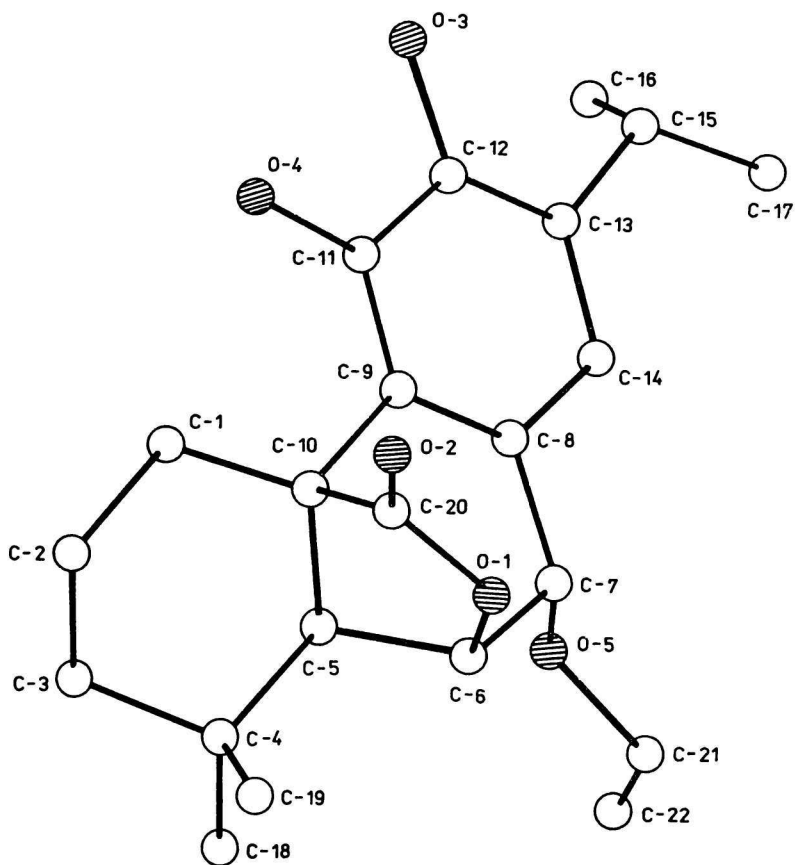
The work-up of ethereal and methanolic extracts afforded eight compounds, which were identified as  $\beta$ -ursolic (*I*) and  $\beta$ -oleanolic (*II*) acids, 7-ethylrosmanol (*III*), cirsimaritin (*IV*), luteolin-7-*O*-glucoside (*V*), apigenin-7-*O*-glucoside (*VI*),  $\beta$ -sitosterol (*VII*), and saccharose (*VIII*).

Compounds *I* and *II* are pentacyclic triterpenes  $C_{30}H_{48}O_3$  (according to mass spectrum) the IR spectra of which differ from each other in the carbonyl and methyl groups absorbance region. Interpretation of the  $^1H$  NMR spectra and their comparison with those of  $\beta$ -ursolic and  $\beta$ -oleanolic acids [14] clearly showed these substances to be identical. Also their m. p.'s were in accordance with those published.



Compound *III*, isolated from the ethereal extract had molecular formula  $C_{22}H_{30}O_5$  ( $m/z = 374$ ). Due to a very low yield of this substance (3 mg) its structure was solved by X-ray crystallographic analysis according to which the compound was identified as 7-ethylrosmanol, a diterpene of abietane type. The compound was previously isolated from *Rosmarinus officinalis* L. [15]. Its presence in the aerial part of *Salvia officinalis* L. was also reported [16].

The structure of 7-ethylrosmanol was confirmed by the plane UV spectrum, the IR spectrum, and the  $^1H$  NMR spectrum. The addition of diagnostic reagents to the  $^1H$  NMR spectrum, which showed signals of protons associated with C-5--OH, two singlets of isolated protons at  $\delta = 6.90$  and 6.81 ppm and two singlets of methoxyl groups at  $\delta = 3.93$  and 3.74 ppm. They were assigned by the NOE differential spectro-



scopy [17]. After presaturating the signal at  $\delta = 3.93$  ppm the NOE effect was observed at  $\delta = 6.90$  ppm, while presaturation of that at  $\delta = 3.74$  ppm remained without effect. Therefore, the methoxyl group resonating at  $\delta = 3.93$  ppm can be located at C-7, and the signal at  $\delta = 6.90$  ppm can be ascribed to C-8 or C-6. Consequently compound *IV* was identified as 5,4'-dihydroxy-6,7-dimethoxyflavone (cirsimaritin).

The UV and the  $^1\text{H}$  NMR spectral data indicate that compound *V* might be luteolin-7-*O*-glucoside. Its  $^{13}\text{C}$  NMR spectrum agreed with that reported for this glycoflavonoid [18]. Hydrolysis of *V* with 2 M-HCl afforded monosaccharide identified respectively

The UV,  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra ascribed the structure of 5,7,4'-trihydroxyflavone glucosylated in position 7.



- IV      $R^1 = R^2 = \text{OCH}_3$ ,  $R^3 = \text{H}$   
 V       $R^1 = \text{O-Glc}$ ,  $R^2 = \text{H}$ ,  $R^3 = \text{OH}$   
 VI      $R^1 = \text{O-Glc}$ ,  $R^2 = \text{H}$ ,  $R^3 = \text{H}$

Comparison of its  $^{13}\text{C}$  NMR data with those of apigenin-7-*O*-glucoside [20] confirmed both substances to be identical; due to a little amount of VI only protonated carbons could be considered. Acid hydrolysis (2 M-HCl) of VI afforded apigenin (the m. p. and UV spectrum were in line with [21]) and a saccharide, which was proved to be glucose as evidenced by chromatography [19].

The remaining two isolated compounds were shown to be  $\beta$ -sitosterol (VII) and saccharose (VIII); physicochemical constants of both were in line with those of the specimens.

## Experimental

*Salvia officinalis* L. was grown in the Experimental Garden of Medicinal Plants, Faculty of Pharmacy, Comenius University in Bratislava. The herb was investigated after being air-dried; the content of essential oil was estimated in the fresh herb.

Melting points were measured on a Kofler micro hot-stage, the mass spectra were recorded with an AEI MS 902 apparatus, the UV and IR spectra with the respective UV VIS (Zeiss, Jena) and Perkin—Elmer (model 477) spectrophotometers. The  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra of pyridine- $d_5$ ,  $\text{CDCl}_3$ , or dimethyl sulfoxide- $d_6$  solutions of compounds containing tetramethylsilane as an internal reference were run with a Bruker AM-300 instrument operating at the frequency of 300 and 75 MHz, respectively. For X-ray analysis diffractometer Syntex P2<sub>1</sub> was employed.

### Extraction and isolation

The dried herb of *Salvia officinalis* L. (800 g) was extracted stepwise with petroleum ether, ether, chloroform, methanol, and dilute methanol ( $\varphi_r = 1/1$ ). The filtered extracts

were separately concentrated under diminished pressure at a water-bath temperature not exceeding 60 °C to dryness. The ethereal macerate containing a mixture of triterpenoids (52.1 g) showing a positive Liebermann—Burchard test was triturated with ether to give a green-white precipitate (31.4 g), which, on crystallization from ethanol yielded  $\beta$ -ursolic acid (*I*) (12.4 g). The mother liquors were chromatographed on a silica gel-packed column (deactivation with 10 % of water) with chloroform and its mixtures with methanol ( $\varphi_r = 98:2$  and  $95:5$ ). The content of fractions (100 cm<sup>3</sup> each) was monitored by thin-layer chromatography on Silufol sheets (detection with sulfuric acid). The combined fractions 22 to 31 were crystallized from ethanol to afford further amount of  $\beta$ -ursolic acid (0.45 g). The combined fractions 69—74 were recrystallized from ethanol to furnish  $\beta$ -oleanolic acid (*II*) (4.5 g). Compounds of the ethereal portion remaining in solution were chromatographed on the same carrier with benzene—ethanol ( $\varphi_r = 99:1$ ) and rising ratios of ethanol. The combined fractions 15—17 (50 cm<sup>3</sup> each) were worked up and crystallized from the mixture hexane—ethanol ( $\varphi_r = 8:2$ ) to give 7-ethylrosmanol (*III*) (3 mg). Similarly, combined fractions 32—48 afforded  $\beta$ -sitosterol (*VII*) (36 mg).

As the qualitative composition of methanolic portions was very close, both were combined and dissolved in water. The undissolved portion was filtered off and the solvent from the filtrate was removed. The residue (10.8 g) was separated on a silica gel-packed column (deactivation with 10 % of water) by elution with chloroform and chloroform—methanol at various ratios. Separated were: circsimaritin (*IV*) (53 mg), luteolin-7-*O*-glucoside (*V*) (68 mg), apigenin-7-*O*-glucoside (*VI*) (12 mg), and saccharose (*VIII*) (3.5 g).

**$\beta$ -Ursolic acid (*I*):** m. p. = 281—284 °C; Ref. [1] gives m. p. = 285 °C. For C<sub>30</sub>H<sub>48</sub>O<sub>3</sub> ( $M_r = 456.3591$ ) found:  $m/z = 456.3603$  ( $M^+$ ), 248 (C<sub>16</sub>H<sub>24</sub>O<sub>2</sub>, base peak), 207 (C<sub>14</sub>H<sub>23</sub>). IR spectrum (KBr),  $\tilde{\nu}/\text{cm}^{-1}$ : 3520 ( $\nu(\text{OH})$ ), 1715 ( $\nu(\text{C}=\text{O})$ ), 1645 ( $\nu(\text{C}=\text{C})$ ), 1030 ( $\nu(\text{C}-\text{O})$ ). <sup>1</sup>H NMR spectrum (pyridine-d<sub>5</sub>, 25 °C),  $\delta/\text{ppm}$ : 5.51 (t, 1H,  $J = 3.6$  Hz, C-12—H), 3.47 (m, 1H, C-3—H), 2.65 (d, 1H,  $J_{18,19\alpha} = 11.2$  Hz, C-18—H), 1.26, 1.25, 1.07, 1.04, 0.91 (5s, 5 × 3H, 5 × CH<sub>3</sub>), 1.02 (d, 3H,  $J_{\text{CH}_3, \text{H}} = 6.5$  Hz, C-19—CH<sub>3</sub>), 0.97 (d, 3H,  $J_{\text{CH}_3, \text{H}} = 6.3$  Hz, C-20—CH<sub>3</sub>).

**$\beta$ -Oleanolic acid (*II*):** m. p. = 300—302 °C; Ref. [22] gives m. p. = 300—302 °C. For C<sub>30</sub>H<sub>48</sub>O<sub>3</sub> ( $M_r = 456.3591$ ) found:  $m/z = 456.3599$  ( $M^+$ ). IR spectrum (KBr),  $\tilde{\nu}/\text{cm}^{-1}$ : 3440 ( $\nu(\text{OH})$ ), 1690 ( $\nu(\text{C}=\text{O})$ ), 1645 ( $\nu(\text{C}=\text{C})$ ), 1030 ( $\nu(\text{C}-\text{O})$ ). <sup>1</sup>H NMR spectrum (CDCl<sub>3</sub>, 25 °C),  $\delta/\text{ppm}$ : 5.28 (t, 1H,  $J = 3.7$  Hz, C-12—H), 3.22 (m, 1H, C-3—H), 2.82 (dd, 1H,  $J_{18,19\alpha} = 13.9$  Hz,  $J_{18,19\beta} = 4.5$  Hz, C-18—H), 1.33, 0.99, 0.93, 0.92, 0.90, 0.78, 0.76 (7s, 7 × 3H, 7 × CH<sub>3</sub>).

**7-Ethylrosmanol (*III*):** m. p. = 220—223 °C; Ref. [15] gives m. p. = 218—223 °C. For C<sub>22</sub>H<sub>30</sub>O<sub>5</sub> ( $M_r = 374.2093$ ) found:  $m/z = 374.2098$  ( $M^+$ ), 330 (C<sub>21</sub>H<sub>30</sub>O<sub>3</sub>), 328 (C<sub>21</sub>H<sub>28</sub>O<sub>3</sub>), 259 (C<sub>16</sub>H<sub>19</sub>O<sub>3</sub>), 215 (C<sub>14</sub>H<sub>15</sub>O<sub>2</sub>), 69 (C<sub>5</sub>H<sub>9</sub>). UV spectrum (methanol),  $\lambda_{\text{max}}/\text{nm}$  ( $\log(\epsilon/(\text{m}^2 \text{mol}^{-1}))$ ): 291 (2.24). X-Ray structural analysis: 7-Ethylrosmanol crystallizes in an orthorhombic system, space group P2<sub>1</sub>2<sub>1</sub>2<sub>1</sub>, number of formula units in the unit cell  $Z = 4$ , lattice parameters  $a = 1.1377(2)$  nm,  $b = 0.8318(1)$  nm,  $c = 2.2077(4)$  nm, volume of the unit cell  $V = 2.082$  nm<sup>3</sup>; the crystal with dimensions 0.4 × 0.2 × 0.2 mm was used for diffraction intensity measurements employing an automated four-circle Syntex P2<sub>1</sub> diffractometer and a graphite monochromated CuK $\alpha$  radiation in a  $\Theta/2\Theta$  to  $\Theta_{\text{max}} = 110^\circ$  mode. Of the total 1533 recorded reflections ( $I \geq 2\sigma(I)$ ) 1454 were classified as

observed and used for the structure determination and refinement. The structure was solved by direct method employing the MULTAN program [23] and refined by the least-squares method in a block-diagonal approximation and weighting scheme  $w = |F_0|/20$  for  $|F_0| < 20$  and  $w = 20/|F_0|$  for  $|F_0| \geq 20$ ; the non-hydrogen atoms were anisotropically refined, the H atoms isotropically, the final values of  $R$  and  $R_w$  were 0.044 and 0.049, respectively. Difference map of the residual electron density did not contain maximum higher than  $150 e \text{ nm}^{-3}$ . Coordinates and thermal coefficients of atoms, bonding and torsion parameters and tables of observed and calculated structure factors are available on request.

*Cirsimaritin (IV)*: m. p. = 262–263 °C; Ref. [21] gives m. p. = 263 °C. UV spectrum (methanol),  $\lambda_{\text{max}}/\text{nm}$  ( $\log(\epsilon/(\text{m}^2 \text{ mol}^{-1}))$ ): 277 (3.43), 336 (3.56); (NaOMe): 275, 294 sh, 390; (AlCl<sub>3</sub>): 264 sh, 294 sh, 301, 365; (AlCl<sub>3</sub>/HCl): 266 sh, 294 sh, 302, 358; (NaOAc): 277, 342, 390; (NaOAc/H<sub>3</sub>BO<sub>3</sub>): 277, 336. <sup>1</sup>H NMR spectrum (dimethyl sulfoxide-d<sub>6</sub>, 40 °C),  $\delta/\text{ppm}$ : 12.89 (s, 1H, C-5—OH), 10.3 (br. s, 1H, C-4'—OH), 7.95 (d, 2H,  $J_{2,3'} = 8.8$  Hz, C-2'—H, C-6'—H), 6.93 (d, 2H, C-3'—H, C-5'—H), 6.90 (s, 1H, C-8—H), 6.81 (s, 1H, C-3—H), 3.93 (s, 3H, C-7—OCH<sub>3</sub>), 3.74 (s, 3H, C-6—OCH<sub>3</sub>).

*Luteolin-7-O-glucoside (V)*: m. p. = 258 °C; Ref. [24] gives m. p. = 248–250 °C. UV spectrum (methanol),  $\lambda_{\text{max}}/\text{nm}$  ( $\log(\epsilon/(\text{m}^2 \text{ mol}^{-1}))$ ): 254 (3.39), 268 (3.35), 348 (3.40); (NaOMe): 263, 300 sh, 394; (AlCl<sub>3</sub>): 275, 298 sh, 328, 432; (AlCl<sub>3</sub>/HCl): 273, 294 sh, 358, 384; (NaOAc): 259, 266 sh, 365 sh, 405; (NaOAc/H<sub>3</sub>BO<sub>3</sub>): 259, 372. <sup>1</sup>H NMR spectrum (dimethyl sulfoxide-d<sub>6</sub>, 30 °C),  $\delta/\text{ppm}$ : 12.97 (s, 1H, C-5—OH), 9.95 (br. s, 1H, C-3'—OH or C-4'—OH), 9.38 (br. s, 1H, C-3'—OH or C-4'—OH), 7.43 (m, 2H,  $J_{2,6'} = 2.1$  Hz, C-2'—H, C-6'—H), 6.91 (d, 1H,  $J_{5,6'} = 8.1$  Hz, C-5'—H), 6.78 (d, 1H,  $J_{6,8} = 2.2$  Hz, C-8—H), 6.74 (s, 1H, C-3—H), 6.45 (d, 1H, C-6—H), 5.37 (br. s, 1H, OH—Glc), 5.09 (br. s, 1H, OH—Glc), 5.07 (d, 1H,  $J_{1''} = 7.3$  Hz, C-1''—H), 5.05 (br. s, 1H, OH—Glc), 4.60 (br. s, 1H, OH—Glc). <sup>13</sup>C NMR spectrum (dimethyl sulfoxide-d<sub>6</sub>, 30 °C),  $\delta/\text{ppm}$ : 164.3 (C-2), 103.1 (C-3), 181.7 (C-4), 156.8 (C-5), 161.0 (C-9), 99.4 (C-6), 162.8 (C-7), 94.6 (C-8), 105.2 (C-10), 121.3 (C-1'), 113.5 (C-2'), 145.7 (C-3'), 149.0 (C-4'), 115.9 (C-5'), 119.0 (C-6'), 99.8 (C-1''), 73.0 (C-2''), 76.3 (C-3''), 69.5 (C-4''), 77.1 (C-5''), 60.6 (C-6'').

*Apigenin-7-O-glucoside (VI)*: m. p. = 220 °C; Ref. [25] gives m. p. = 218 °C. UV spectrum (methanol),  $\lambda_{\text{max}}/\text{nm}$  ( $\log(\epsilon/(\text{m}^2 \text{ mol}^{-1}))$ ): 266 (3.63), 332 (3.70); (NaOMe): 268 sh, 303 sh, 390; (AlCl<sub>3</sub>): 277, 303, 346, 382; (AlCl<sub>3</sub>/HCl): 278, 303, 345, 384; (NaOAc): 256 sh, 270, 358, 387; (NaOAc/H<sub>3</sub>BO<sub>3</sub>): 267, 333. <sup>1</sup>H NMR spectrum (dimethyl sulfoxide-d<sub>6</sub>, 25 °C),  $\delta/\text{ppm}$ : 12.95 (s, 1H, C-5—OH), 10.4 (br. s, 1H, C-4'—OH), 7.96 (d, 2H,  $J_{2,3'} = 8.7$  Hz, C-2'—H, C-6'—H), 6.94 (d, 2H, C-3'—H, C-5'—H), 6.89 (s, 1H, C-3—H), 6.83 (d, 1H,  $J_{6,8} = 2.1$  Hz, C-8—H), 6.45 (d, 1H, C-6—H), 5.42 (br. d, 1H, OH—Glc), 5.14 (br. d, 1H, OH—Glc), 5.09 (br. d, 1H, OH—Glc), 5.06 (d, 1H,  $J_{1'',2''} = 7.3$  Hz, C-1''—H), 4.64 (br. t, 1H, C-6''—OH). <sup>13</sup>C NMR spectrum (dimethyl sulfoxide-d<sub>6</sub>, 40 °C),  $\delta/\text{ppm}$ : 128.6 (C-2', C-6'), 116.0 (C-3', C-5'), 103.1 (C-3), 99.9 (C-1''), 99.5 (C-6), 94.8 (C-8), 77.1 (C-5''), 76.4 (C-3''), 73.1 (C-2''), 69.6 (C-4''), 60.7 (C-6'').

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