SUPPLEMENTARY MATERIAL

Phytoecdysteroids-containing extract from *Stachys hissarica* plant and its wound healing activity

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Abstract

A number of phytoecdysteroid compounds, such as ecdysterone, polipodin V, 2-deoxy-20-hydroxyecdysone, integristeron A, and 2-deoxydizon were isolated from *Stachys hissarica* plant and their structures were confirmed by NMR-, mass- and IR- spectroscopy. In addition, the biological activity of the *Stachys hissarica* plant's extract was tested on rats for wound healing activity. It was shown that the extract at repeated oral (per os) administration at a dose of 10 mg/kg speeds up the healing process of linear skin wounds in rats. The wound healing activity of *Stachys hissarica* extract is confirmed to be effective and exceeds known drug methyluracil (2,4-dioxo-6-methyl-1,2,3,4-tetrahydropyrimidine), especially in case of alloxan induced diabetic animals.

Keywords: phytoecdysteroids, *Stachys hissarica*, ecdysterone, wound healing activity, extract

EXPERIMENTAL PART

Chemical evaluation

UV-spectra were measured on Lambda-16 spectrophotometer (Perkin Elmer). The melting point for investigated substances was measured by Boetius (Germany) and Mel-Temp (USA). IR-spectra were recorded using FT-IR spectrometer Perkin Elmer, Model 2000 (KBr disc), Nuclear Magnetic Resonance spectra ^1H and ^{13}C were recorded using VN MRS-400 (Varian), with working frequency of 400 MHz. The samples were dissolved in Py-d_5 solvent, internal standard is hexamethyldisiloxane (HMDS). For TLC were used chromatographic plates Silufol UV 254, for plates development was used 3% solution of vanillin in ethanol, within solvent systems chloroform-methanol 1, 9:1; 2, 4:1; (system A), and chloroform-methanol-water 1, 4:1:0.1; 2, 70:23:3 (system B).

In this work, for thin-layer and column chromatography the silica gel of types "KSK", "L" (Czech Republic) and aluminum oxide were used. TLC was conducted on the fixed layer of silica gel, sieved through a sieve with 0.08 mm mesh size, containing 7% of gypsum on the plates «Silufol». For column chromatography the silica gel with a particle size of 0,1-0,16 mm and 0.08-0.1 was used.

20-hydroxyecdysone (1), $\text{C}_{27}\text{H}_{44}\text{O}_7$, m.p. 241-242 °C (recrystallized from acetone), $[\alpha]_{\text{D}} +63.2 \pm 2^\circ$ (with 6.30; methanol). UV-spectrum ($\text{C}_2\text{H}_5\text{OH}$, $\lambda_{\text{max}} > \text{nm}$): 245 nm ($\text{Ig} \epsilon 4.01$), IR-spectrum (KBr, ν , cm^{-1}): 3435 (OH), 1665 (7-en-6-keto group).

Mass spectrum, m/z (%): 480 (M; 0.03), 462 (1), 444 (2), 446 (14), 11 (4), 408 (10), 393 (5), 363 (10), 345 (33), 327 (20), 301 (16), 300 (12), 161 (5), 143 (10), 125 (8), 107 (6), 99 (100), 81 (34), 69 (21).

The comparison of the obtained data and spectral characteristics [Ramazanov 2007b] with data from literature has allowed to identify the ecdysteroid.

Polipodin B (2), $\text{C}_{27}\text{H}_{44}\text{O}_8$, after recrystallization from acetone had m.p. 252-254 °C, $[\alpha]_{\text{D}} +94.2 \pm 2^\circ$ (with 0.50; methanol). The IR-spectrum (KBr, ν , cm^{-1}): 3400 (OH), 1673 (7-en-6-ketogrouping). The UV-spectrum ($\text{C}_2\text{H}_5\text{OH}$, $\lambda_{\text{max}} > \text{nm}$): 244 nm ($\text{Ig} \epsilon 3.9$).

Mass spectrum, m/z (%): 478 (M-H₂O; 0.03), 463 (0.1), 460 (0.2), 445 (0.3), 427 (0.4), 424 (0.4), 409 (0.7), 379 (6), 361 (100), 360 (86), 344 (4), 343 (4), 325 (5), 316 (10), 99 (12), 81 (13), 69 (15).

The structure of compound 2 was confirmed by spectral data and by comparison with original sample on thin layer chromatography [Ramazanov 2007b].

2-dezoxy-20-hydroxyecdysone (3), C₂₇H₄₄O₆, m.p. 254-256 °C (from water ethanol), [α]_D²³+82.0±3° (with 1,3; methanol). λ₂₅^{C₂H₅OH}_{max}:246 nm (lg ε 4,10); the IR-spectrum (KBr, ν, cm⁻¹): 3370 (OH), 1645 (7-en-6-keto group) [Ramazanov 2007a].

Mass spectrum; m/z (%): 464 (M+, 1), 446 (3), 428 (10), 413 (8), 410 (14), 395 (6), 392 (6), 377 (5), 347 (35), 329 (90), 312 (33), 311 (28), 303 (9), 302 (11), 297 (13), 295 (14), 285 (30), 284 (35), 234 (15), 233 (15), 99 (100), 81 (73).

Integristeron A (4), C₂₇H₄₄O₈, with m.p. 246-248 °C (from mixture of ethyl acetate-methanol), [α]_D²⁰ +36.1±2 ° (from 0.43; methanol). UV spectrum (C₂H₅OH, λ_{max}> nm): 245 nm (lg ε 4.00). IR spectrum (KBr, ν, cm⁻¹): 3400 (OH), 1660 (7-en-6-keto grouping).

Mass spectrum, m/z (%): 478 (M-H₂O; 3), 460 (4), 445 (10), 442 (18), 427 (14), 409 (7), 391 (4), 379 (73), 374 (5), 368 (17), 361 (74), 343 (100), 325 (43), 316 (17), 309 (7), 283 (41), 143 (60), 135 (61), 99 (35), 81 (34).

¹H NMR (C₅D₅N; δ, ppm; 0-TMS): 1.19 (3H at C-18,); 1.40 (3H at C-19,); 1.39 (6H at C-26/27,); 1.58 (3H at C-21,); 3.57 (1H at C-9); 3.77 (1H at C-22,); 4.30 (3H at C-1,2,3,); 6.17 (1H at C-7, br. s).

The spectral data and comparison with the original sample (by TLC) have allowed to identify the integristeron A [Shishkin 1963].

2-dezoxy-α-ecdysone (5), C₂₇H₄₄O₅, m.p. 235-236 °C (from water-ethanol), IR-spectrum (KBr; ν, cm⁻¹): 3429 (OH), 1662 (7-en-6 keto group).

Mass spectrum; m/z (%): 448 (M 2), 430 (13), 415 (14), 412 (11), 402 (16), 285 (12), 284 (12), 235 (27), 234 (50), 233 (32), 99 (100), 81 (63).

Biological evaluation

This experiment was performed under the approval of the State Committee on Animal Ethics, ICPS, Tashkent (Uzbekistan). Also, the recommendations of the European Council

Directive (86/609/EC) of November 24, 1986, regarding the protection of animals used for experimental purposes were considered.

Normal and alloxan-induced diabetic male rats with weight of 150-160g were used in the experiment. The experimental diabetes condition was induced by hypodermic administration of alloxan in a dose of 170 mg/kg. The animals were participating in experiment on the 21st day after the administration of toxicant (blood sugar within 280-320 mg/%).

Linear skin wounds were inflicted under a slight anesthesia. For this purpose the incision of 3 cm long was made on the back midline (fur was removed), on which two sutures on equal distance were placed to pull together wound edges. The tensile strength measurement was applied to control the healing process and to define the mechanical properties of postoperative scar [Misharev 1984]. The estimation of wound healing activity of TEM from *Stachys hissarica* was performed on the 10th day of the experiment (after removal of seams), the extract was administered *per os* in a dose of 10 mg/kg throughout the experiment.

The same way was administered into rats methyluracil, 2,4-dioxo-6-methyl-1,2,3,4-tetrahydropyrimidine in a dose of 50 mg/kg, which was a reference compound in the experiment. This compound possesses an anabolic activity, accelerates the processes of cellular regeneration, and is effective in poorly healing wounds [Mashkovskiy 1998].

REFERENCES

- Mashkovskiy MD. 1998. The Drugs, Medical literature publishing named after Abu Ali Ibn Sino, 2:168–169.
- Misharev OS, Abaev, JK, Prokopchuk NR. 1984. An estimation of the strength of wound edges healing, Vestnik khirurgii, 133:82–85.
- Ramazanov NS. 2007a. The plants of ecdysteroids from *Silene*, *Rhapontcium* and *Ajuga*. Tashkent: Uzbekistan.
- Ramazanov NS, Mamadaliyeva NZ, Bobaev ID. 2007b. Phytoecdysteroids from five species of the genus *Silene*, Chem Nat Comp, 43:117-118.
- Shishkin BK. 1963. Flora of the USSR. Vol. 28. Science. Moscow-Leningrad.