ХИМИЯ РАСТИТЕЛЬНОГО СЫРЬЯ. 2021. №1. С. 197–202.
DOI: 10.14258/jcprm.2021018323

UDC 547.913:543.062

BIOLOGICAL ACTIVE COMPONENTS OF *SILENE TOMENTELLA* AND THEIR PHARMACOLOGICAL PROPERTIES

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In thepresent investigation isolation of chemical compounds was carried out from the aerial part of *Silene tomentella*, an evergreen member of the family *Caryophyllaceae*, using column chromatography. Identification of chemical compounds was done by various MP, TLC, IR, NMR techniques. The presence of the bioactive compound D-pinitol in this plant is being reported for the first time. Under the influence of D-pinitol, a clear tendency towards normalization of the glycogen content in the liver was also noted (it was only 10.4% lower than in intact animals). In the liver of animals, a rather sharp decrease in the content of glycogen was noted – by 63.5%, and a decrease in the activity of enzymes of the antioxidant defense of the body, characteristic of developing diabetes: SOD – by 35.1, and catalase – by 32.4%. Prophylactically – therapeutic administration of D- pinitol, which exhibits a pronounced ability to inhibit free radical oxidation processes.

Keywords: Silene tomentella, D-pinitol, hypoglycemic and antioxidant activities, phytoecdysteroids (PE).

The project was funded through a grant from the Republic of Uzbekistan State Foundation [grant number PZ-20170927342]

Introduction

Currently, main issue from scientific and practice sight is obtaining drugs from plant source in order to providing medicine for public health. In the world research work carries out to find alternative source active compound from plants with a deep understanding biological impact and to extend exist source of effective, eco-friendly and economical beneficial medicine [1]. Moreover, study of the most widespread traditional medicine of medicinal plants in Uzbekistan with the aim of introducing them into medical practice [2].

Attention to the *Caryophyllaceae* Juss. family increased substantially because of the discovery of the molting hormones of insects. This plant family includes (according to Takhtadzhyan (1987)) 80 genera and 2100 species, thus it is one of the richest ecdysteroid-containing families in the world flora. At present, according to literature data and our experimental results, ecdysteroids were discovered in more than 150 species of the *Silene* L. genus [3]. So, the caryophyllaceous family is rich in the species (particularly genus *Silene*) able to synthesize ecdysteroids in large amounts and in a broad ecdysteroid profile [4, 5]. However, this family is numerous and insufficiently studied yet, which attracts our attention and the attention of other researchers.

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Ecdysteriods are arthropod steroid hormones controlling molting (ecdysis), development and reproduction through interaction with the ecdysteroid receptors. To date, more than 500 ecdysteroids have been characterized [6]. 20-Hydroxyecdysone (20E, alsocommonly reported as ecdysterone, β-ecdysterone and 20-hydroxy-α-ecdysone) is the most prevalent andabundant phytoecdysteroid produced by plants, and a major component in phytoecdysteroidcontaining herbal extracts [5, 7–9]. Phytoecdysteroids are at-

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tributed with numerous otherpharmacological properties in animals, including humans. They have been shown to stimulatecarbohydrate metabolism and reduce hyperglycemic response in rats and mice and decreaseweight gain in diet-induced obese mice, which have significant implications for reducingdiabetes symptoms in humans [10–12].They have also been used to restore renal dysfunction and fortreatment of cardiovascular disease [13, 14].

The compounds pinitol is belonging to group of Cyclitols (cyclic polyol). Pinitol (3-O-methyl D-chiro inositol) is a natural product of cyclitol group occurring mainly in its (+) form in certain leguminous plants, soya foods and was found to be responsible for hypoglycaemic activities, antidiabetic & its chronic complications obesity; Hyperlipidemia; Dyslipidemia atherosclerosis; Hypertension; cardiovascular disease, malnutrition, stress, aging & other autoimmune disease, Hyperuricemia & Anthelmintic activity [15, 16]. (+)-pinitol has been isolated and characterized from the bark of this medicinally important tree. Pinitol is a well known bioactive compound for a variety of biological activities, including hypoglycemic, antioxidant and anti-inflammatory activities [17]. Herbal medicine is the oldest form of health care known to mankind. Herbs had been used by all cultures through history [18]. There is a growing interest in the use of D-pinitol as a food supplement because of its reported efficacy in lowering blood glucose levels with no side effects and nil toxicity [19]. D-pinitol is used as a hypoglycemic and antidiabetic medicine in the USA [20].

The representatives of the numerous *Silene* genus*,* distinguished by the diversity of structures and the high level of ecdysteroids [21].

Ecdysteroids in *S.tomentella* were discovered for the first time [22, 23] with the help of the developed method based on the chromatographic analysis. *S.tomentella* (Regel) a wild medicinal plant indigenous to Central Asia, was prioritized for botanical investigation because it is valued for its purported adaptogenic properties [24]. “Adaptogenic” refers to the ability of a compound to enhance physical strength and stamina as well as increase nonspecific resistance of an organism without interference with normal biological functions [25].

S. tomentella accumulates of plant secondary compounds known as phytoecdysteroids and D-pinitol. Furthemore, D-pinitol (more 0.1% in air-dried material), which has hypoglycemic and anti-oxidative properties.

Experimental part

General remarks. IR spectra were recorded on a System 2000 Fourier spectrometer (Perkin Elmer) in tablets with KBr. NMR spectra were recorded on a Unity 400 Plus spectrometer (Varian) with an operating frequency of 400 MHz. Melting points of the isolated compounds were determined in glass capillaries on an A. KRUSS OPTRONIC instrument (Equipment, Germany). For TLC analysis, chromatographic plates "Silufol UV 254" were used. For manifestation the plates used vanillic acid, UV lamp light at 254 nm and 365 nm.

Plant material. Silene tomentella plant was collected in 2018 in the mountains of Kulzhuktau, Bukhara region of the Republic of Uzbekistan during the growing season under the supervision of Dr. A.M. Nigmatullaev. An authenticated voucher sample (Speech No. 1218) of this plant species is stored in a herbarium (Institute of the Chemistry of Plants substances).

Extraction and Isolation. The dried and ground aerial part of *S. tomentella* (1.0 kg) was extracted four times with methanol (MeOH) (4×6 L) at room temperature exhaustion (no considerable yield was further obtained), i.e. the extraction was carried out four times, using 6 L of neat methanol each. The solvent was evaporated at 40^{o}C using a rotary vacuum evaporator (Heidolph Instruments GmbH & CO. KG, Germany). The extract was concentrated and diluted with an equal volume of water. The aqueous portion was sequentially extracted with chloroform to separate plant pigments (such as chlorophyll, carotenoid and etc.), with ethyl acetate to reduce a less polar compounds, and finally with *n*-butanol. After evaporation of the solvents in vacuum, a butanol fraction was obtained. The butanol fraction was evaporated on a rotary evaporator and the crude extract (10 g) was subjected to silica gel (200–300 mesh) column chromatography, eluting with a CH_3Cl_3-CH_3OH gradient system (100 : 1, 60 : 1, 50 : 1, 30 : 1, 20 : 1, 15 : 1, 9 : 1, 4 : 1), to give 10 fractions, 1–10. Further separation of fraction 7–10 (50 : 1, 25.4 g (6.5, 5.5, 6.3, 7.1 g)) by silica gel column chromatography, eluted with CH_3Cl_3-CH_3OH (50 : 1), to give compound D-pinitol (1) (1.0 g). continuous yielded with CH_3Cl_3-CH_3OH (30 : 1, 20 : 1, 15 : 1, 9 : 1, 4 : 1), to obtained compounds 20-hydroxyecdysone (2) (11 mg), cyasterone (3) (8 mg), α-ecdysone (4) (5.1 mg).

Experimental animals. This study was performed using male rats with body weight of 170-190 g. Rats were kept in one polyacrylic cage and all the rats were quarantined for 1 week before the experiments. All animals housed under standard controlled conditions with temperature at 24±2 °C, humidity of 50±5% and 12 h light/dark cycle with free access to food (standard commercial rat chow) and water, and received care according to European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes (Strasbourg, 1986).

Biological activity. D-pinitol was administered to animals (male rats, 150–180 g) orally at a dose of 50mg/kg. Its effect on the level of glycemia was evaluated in experiments on intact (normal) rats and rats with developing alloxan diabetes (alloxan was injected subcutaneously once at a dose of 150 mg/kg). In the latter case, the animals were taken into the experiment 7 days after the administration of the selected toxicant. The hypoglycemic and anti-diabetic effects of pinitol were assessed using the glucose tolerance test (GTT): 5000 mg/kg glucose orally as a 20% solution. Blood for research in rats was taken from the tail vein 30 minutes after it entered the body (the period corresponding to the peak of the "sugar curve" in rats in our experiments). The blood sugar content was determined using the enzyme-colorimetric method using Cypress diagnostics kits (Belgium) on a Secomam Basic biochemical analyzer (France). Insulin content was determined by enzyme immunoassay with COBAS reagents (Roche Diagnostics GmbH). The study of the effect of pinitol on the glycogen content in the liver was carried out according to the method [26]. The presence of antioxidant properties in pinitol when it is introduced into the body of animals was judged by the amount of malondialdehyde (MDA), the activity of superoxide dismutase (SOD) and catalase in the liver of rats, respectively, according to the methods [27–30]. In intact animals, pinitol was administered 2.5 hours before glucose injection. The same was done in alloxan-diabetic rats with the only difference that pinitol began to be administered simultaneously with alloxan and was administered for 6 days prior to HTT. During the experiments, all animals were kept under standard vivarium conditions on a regular diet with free access to water. Experiments with them were carried out in accordance with the rules adopted by the International Convention for the Protection of Vertebrate Animals used for experimental and other scientific whole (Strasbourg, 1986).

Results and discussion

As a result, it was found that the butanol extract of the aerial part of *S. tomentella* contains ecdysteroids, the medium of which is ecdysterone (2).

As a result of chromatographic separation of ecdysteroid fractions of one stripped off water-alcohol and n-butanol extracts from the aerial part of the plant, a target white fine-crystalline (powder) non-steroidal substance was isolated (1).

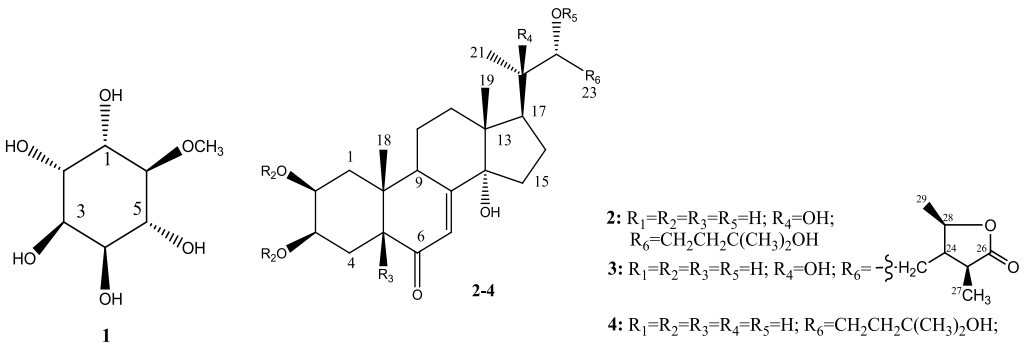
Isolated individual components: D-pinitol (1), 20-hydroxyecdysone (2), cyasterone (3), α-ecdysone (4). Isolated individual compounds have been identified on the basis of the ^{1}H, ^{13}C NMR and TLC, as well as by comparison with reference compounds (see supplementary information). The structures of phytoecdysteroids are shown below, i.e., their names (Fig.).

D-pinitol (1). The gross formula is $C_7H_{14}O_6$ $[\alpha]^{D}_{20}$+65.5°. Yield 1.0 g (0.1%, dry weight), white shiny small crystals. M.p. 185 °C. In the IR spectrum of substance (1), there are bands of stretching vibrations of hydroxyl groups in the region of 3650–3300 cm^{-1}, aliphatic C-C bonds 2370–2900 cm^{-1} and C-O bonds 1170 cm^{-1}, and in the UV spectrum and there are no absorption bands in the visible region.

The ^{1}H-NMR spectrum of compound (1) contains signals of 14 protons, as well as residual DMSO signals.

All signals of the protons of a substance are in the range of 2.9–4.7 ppm, which indicates the presence of only aliphatic atoms:^{1}HNMR (400 MHz, DMSO-d_6, δ, ppm., J/Hz): 2.95 (1H, t, *J*= 9.3, H-6), 3.28 (1H, td, *J*= 9.4, 4.8, H-5), 3.39 (3H, s, OCH_3), 3.39 (1H, m, H-4), 3.45 (1H, ddd, *J* = 9.2, 6.4, 2.3, H-1), 3.57 (2H, m, H-2, H-3), 4.33 (1H, d, *J*= 5.8, 4-OH), 4.45 (1H, d, *J*= 6.5, 1-OH), 4.50 (1H, d, *J*= 4.8, 5-OH), 4.60 (1H, d, *J*= 3.0, 3-OH), 4.69 (1H, d, *J*= 2.9, 2-OH). Substance 1 was identified by spectral data ^{1}H NMR with authentically D-pinitol sample [31].

^{13}C-NMR spectrumrevealed signals from seven carbon atoms, which connected oxygen atoms in the range of (70.17–83.89 ppm) , and one carbon atom of the methoxyl group at 59.79 ppm.



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Chemical structures of phytoecdysteroids isolated from S. tomentella

^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 70.17 (C-1), 72.69 (C-2), 72.06 (C-3), 70.98 (C-4), 72.51 (C-5), 83.89 (C-6), 59.79 (OCH_3). Based on the study of spectral data and comparison with the original sample substance **1** was identified with D-pinitol [32].

20-Hydroxyecdysone (2). White crystals, m.p. 243 °C (from acetone), $[\alpha]^D_{23}$+63.2±2° (from 6.30, methanol). UV spectrum (C_2H_5OH, λmax, nm): 245 (Igε 4.01). IR spectrum (KBr, ν, cm^{-1}): 3433 (OH), 1666 (7-en-6-keto group). Yield 11 mg, 0.0011%.

Substance 2 was identified by spectral data and direct comparison on TLC with authentically 20-hydroxyecdysone sample [4].

Cyasterone (3). $C_{29}H_{44}O_8$, white powder, mp 156°C, $[\alpha]^D_{23}$+60.0±2° (c 1.0, pyridine). Yield 8 mg, (0.0008% dryweight). ^{13}C NMR (100 MHz, C_5D_5N, δ, ppm): 39.22 (C-1), 68.62 (C-2), 68.56 (C-3), 32.96 (C-4), 51.90 (C-5), 203.96 (C-6), 122.36 (C-7), 166.34 (C-8), 38.48 (C-9), 40.42 (C-10), 21.87 (C-11), 32.57 (C-12), 48.70 (C-13), 84.67 (C-14), 32.42 (C-15), 21.50 (C-16), 49.21 (C-17), 18.43 (C-18), 24.96 (C-19), 77.31 (C-20), 21.62 (C-21), 74.51 (C-22), 34.99 (C-23), 50.53 (C-24), 42.96 (C-25), 179.72 (C-26), 16.43 (C-27), 80.36 (C-28), 19.86 (C-29). UV and IR spectra are identical to those given in [5].

α-Ecdysone (4). White crystals (EtOH-H_2O), m.p. 234 °C, $[\alpha]^D_{23}$ + 93.3±2 (c 0.5, MeOH). IR spectrum (KBr, ν, cm^{-1}): 3469 (OH), 1641 (7-en-6-keto group). Yield 5.1 mg, 0.00051%.

Substance 4 was identified by spectral data and direct comparison on TLC with authentically sample of α-ecdysone [4].

Biological activity. Earlier it was shown that pinitol has a certain antihyperglycemic effect [16]. However, many aspects of the related effects of this compound remained unclear. In this regard, experiments were carried out to study its hypoglycemic activity both in normal rats and in rats with experimental insulin-dependent diabetes caused by alloxan [33] using the glucose tolerance test (GTT).

Studies have shown that when the glucose tolerance test was performed in normal animals, the tolerance to the introduction of glucose in the case of using pinitol was significantly increased. Blood sugar in animals receiving pinitol before glucose load increased only by 21.2% compared to the initial one, while in the group of rats that did not receive pinitol, this increase was 56.6%. An insulinemic reaction to glucose administration was also characteristic. In the group of rats that received the test substance, the insulin content increased by 48.7%; those who did not receive pinitol, the insulin content in the blood plasma increased by 95.4%. Changes in liver glycogen content corresponded to changes in blood glucose (Tab.).

The introduction of exogenous glucose into intact rats, leading to a sharp increase in its blood content in the experiments performed, was practically not accompanied by significant changes in the content of MDA, SOD, and catalase in the liver. However, if animals received pinitol during GTT, the MDA content (in relation to the indices of intact animals) decreased by 19.5%, while the activity of SOD and catalase increased by 22.3 and 19.1%, which indicated that pinitol had an antioxidant effect. The work [34] is also of some interest in this regard.

Effect of pinitol on blood glucose and insulin, as well as glycogen, malondialdehyde, superoxide dismutase and catalase in the liver of intact and alloxan-diabetic rats (M±m, n=6–8)

The studied indicators	Intact rats			Rats 7 days after administration of alloxan		
	Initial value (control I)	GTT	GTT+D-pinitol	Initial value (control II)	GTT	GTT+D-pinitol
Blood						
Glucose, mM/l	4.80±0.20	7.52±0.44[1]	5.82±0.36[1,2]	11.6±0.56[1]	21.2±2.12[3]	12.6±0.64[5]
Insulin, μU/ml	30.8±2.12	60.2±4.74[1]	45.8±3.18[1,2]	16.4±1.18[1]	19.4±1.32	35.6±2.40[4,5]
Liver						
Glycogen, mg%	1920±58.2	2280±74.6[1]	1988±60.4[2]	692.4±52.6[1]	620.4±46.4	996.0±64.2[4,5]
MDA, nmol/mg protein	0.472±0.024	0.480±0.026	0.380±0.017[1,2]	0.756±0.042[1]	0.982±0.064[3]	0.542±0.022[4,5]
SOD, PU min/mg/ protein	0.752±0.048	0.710±0.032	0.920±0.040[1,2]	0.488±0.016[1]	0.382±0.012[3]	0.614±0.038[4,5]
Catalase, mkat/min/g protein	13.6±0.42	13.2±0.32	16.2±0.48[1,2]	9.2±0.14[1]	7.6±0.12[3]	11.2±0.34[4,5]

Note: 1 – Reliable to the corresponding indicators in intact animals (control I), 2 – significantly between the corresponding indicators in intact rats under GTT conditions in the groups who did not receive and received pinitol, 3,4 – significantly to the corresponding indicators in alloxan-diabetic rats (control II); 5 – significantly between the corresponding indicators in alloxan-diabetic rats under HTT conditions in the groups that did not receive and received pinitol (the level of reliability was taken at $p<0.05$).

When rats were injected with alloxan, already after 7 days, the basal glucose level (compared to this indicator in intact animals) was increased by 141.7%, while the insulin level was reduced by 46.7%. In the liver of animals, a rather sharp decrease in the content of glycogen was noted - by 63.5%, and a decrease in the activity of enzymes of the antioxidant defense of the body, characteristic of developing diabetes: SOD – by 35.1, and catalase – by 32.4%. All these changes occurred against the background of a sharp activation of free-radical oxidation processes, which was indicated by an increase in the amount of MDA by 60.2%.

It is well known that alloxan is selectively metabolized by β-cells of the pancreas with the release of toxic free radicals that promote the development of alteration processes in them [35, 36]. Therefore, it is not surprising that the prophylactic – therapeutic administration of pinitol, which exhibits a pronounced ability to inhibit the processes of free radical oxidation (Tab.), largely prevents the development of an insulin deficiency state when alloxan enters the body.

In the group of rats with developing alloxan diabetes, the glucose tolerance test, on the one hand, revealed deeper negative changes in carbohydrate metabolism and the course of lipid peroxidation processes, on the other hand, it showed an even more pronounced effectiveness of pinitol as a hypoglycemic agent, which also has a pronounced inhibitory effect. on LPO processes in the body. So if in this series of experiments (Tab.) in rats after the introduction of exogenous glucose the level of glycemia increased by 82.7%, then the previous administration of pinitol led to an increase in glycemia only by 8.6% (in relation to the initial indicators of the corresponding values determined for rats of this series). The table also shows that in rats of this series of experiments, a sharp increase in blood sugar during GTT was not accompanied by a significant increase in insulin content in blood plasma (also in relation to the initial values determined for rats of this series), the increase was only 18.3%. Pinitol, showing a pronounced hypoglycemic effect, had a positive effect on the function of the insular apparatus of the pancreas in rats with developing alloxan diabetes (which, apparently, played a decisive role in this regard). Under its influence, the insulin content in blood plasma during HTT increased by 117.1%. Under the influence of pinitol, a clear tendency towards normalization of the glycogen content in the liver was also noted (it was only 10.4% lower than in intact animals).

Conclusion

Substances (1), (3) and (4) were first isolated from aerial part of *S. tomentella*. It should be noted that due to the high content of D-pinitol (1.0 g (0.1%) based on the weight of air-dry raw materials), which has antidiabetic and antioxidant properties. In addition, the stimulating effect of pinitol on the activity of SOD and catalase enzymes also seems to be an extremely important point in the mechanism of its action, since an increase in the antioxidant defense enzymes of the body significantly inhibits the processing of diabetes and many of its complications (including vascular) arising from increased development in this the case of oxidative stress processes.

Disclosure Statement

No potential conflict of interest was reported by the authors.

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Received August 19, 2020

Revised December 10, 2020

Accepted December 23, 2020

For citing: Yusupova U.Yu., Ramazonov N.Sh., Bobakulov Kh.M., Egamova F.R., Syrov V.N., Usmanov D.A. *Khimiya Rastitel'nogo Syr'ya*, 2021, no. 1, pp. 197–202. DOI: 10.14258/jcprm.2021018323.