Phytoecdysteroids of the East Asian *Caryophyllaceae*

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ABSTRACT

Background: Occurrence of integristerone A (1), 20-hydroxyecdysone (2), ecdysone (3), 2-deoxy-20-hydroxyecdysone (4) has been analyzed in 64 species of the East Asian *Caryophyllaceae.* **Materials and Methods:** Ecdysteroid content was determinate by high-performance liquid chromatography (HPLC). HPLC with a high-resolution mass spectrometry was performed on Shimadzu LCMS-IT-TOF (Japan) system equipped with a LC-20A Prominence liquid chromatograph, a photodiode array detector SPD-M20A and ion-trap/time-of-flight mass spectrometer. **Results:** New sources of phytoecdysteroids: *Melandrium sachalinense* and *Melandrium firmum* have been revealed. It is the 1st time that two has been identified in *M. sachalinense* and *M. firmum*; 1 in the species: *Lychnis fulgens, Silene repens, Silene foliosa, Silene stenophylla, Silene jenisseensis* and *M. sachalinense*; 3 in *Lychnis cognata*; 4 in *L. fulgens, S. stenophylla* and *S. jenisseensis* (the tribe *Lychnideae*, the subfamily *Caryophylloideae*). Ecdysteroid-negative taxa are *Spergularia rubra* of the tribe *Sperguleae*; species of the genera *Minuartia, Honckenya, Eremogone, Arenaria, Moehringia, Pseudostellaria, Fimbripetalum, Stellaria* and *Cerastium* of the tribe *Alsineae*; *Scleranthus annuus* of the tribe *Sclerantheae*, as well as the East Asian representatives of the genera *Gypsophila, Psammophiliela*, *Dianthus* and *Saponaria* of the tribe *Diantheae*; *Oberna* and *Agrostemma* of the tribe *Lychnideae*. **Conclusion:** This investigation shows the most promising sources of ecdysteriods are species of genera *Silene* and *Lychnis*.

Key words: *Caryophyllaceae,* ecdysteroids, high-performance liquid chromatography

INTRODUCTION

Plants of the family *Caryophyllaceae* are perspective sources of phytoecdysteroids-hormones which control molting in insects and crustaceans. Phytoecdysteroids possess stimulating and adaptogenic action, causing considerable decrease in cholesterol content in blood serum and displaying anabolic activity in relation to human and mammals; in this case, unlike anabolic steroids, ecdysteroids, possessing pronounced anabolic activity, do not manifest androgen effect to make possible their lengthy use. Their use in medicinal preparations of adaptogenic, cardiotropic, antiatherosclerotic, counter-ulcer, would-healing and antimicrobial action was shown to be promising.[1-3]

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First studies on phytoecdysteroids of the *Caryophyllaceae* in the flora of the former Soviet Union were conducted by the researchers of the Institute of the Chemistry of Plant Substances of the Academy of Sciences of the Uzbek SSR (Tashkent city) in the 1980s.[4-9]

New sources of phytoecdysteroids are actively sought. Distribution pattern of integristerone A (1), 20-hydroxyecdysone (2), ecdysone (3), 2-deoxy-20-hydroxyecdysone (4) in plants is studied.[10-13]

MATERIALS AND METHODS

Chemicals

Standards of compounds 2, 3, and 4 were obtained from Sigma and Aldrich Company (St. Louis, MO, USA). Compound 1 was obtained from U. A. Baltaev (Institute of

Petroleum Chemistry and Catalysis, Ufa, Russian Federation). All solvents and chemicals were of analytical grade.

Plant materials

The plant material from wild flora was collected during 2005-2012 years in the Far East, Russian Federation. Specimens have been deposited in the herbarium of the laboratory of plant chemotaxonomy of G. B. Elyakov Pacific Institute of Bioorganic Chemistry of the Far-Eastern Branch of the Russian Academy of Sciences. Ecdysteroid content was analyzed in the aerial part of plants collected during their flowering period.

Analysis of ecdysteroids

Air-dried samples (about 200 mg, accurately weighed, residual moisture <8.2%) were extracted with ethanol-water (7:3, 10 mL) at room temperature for 3 days. The resulting extracts were filtered. A portion (0.9 mL) was treated with water (12 mL). Solid-phase extraction was carried out with Supelclean C18 columns (Supelco, St. Louis, USA) using ethanol-water (3:2) as eluent.

High-performance liquid chromatography (HPLC) with a high-resolution mass spectrometry was performed on LCMS-IT-TOF (Shimadzu, Kyoto, Japan) system equipped with a LC-20A Prominence liquid chromatograph, a photodiode array detector SPD-M20A (Shimadzu, Kyoto, Japan) and ion-trap/time-of-flight mass spectrometer. Separation occurred over a column - Ascentis C_{18} 100 × 2.1 mm i.d.; 3.0 μm part size, Supelco, USA) at 40°C. Elution rate was 0.25 mL/min. The elution gradient was as follows: Acetonitrile-water (1:9, v/v) 5 min, acetonitrile-water (1:1, v/v) 15 min, acetonitrile 15 min. The range of detection was m/z 200-800 (atmospheric pressure chemical ionization [APCI], positive ion detection). The potential in the ion source was - 4.5 kV. The drying gas (N_2) pressure was 25 kPa. The nebulizer gas (N_2) flow rate was 2 L/min. The interface temperature was 350°C. Phytoecdysteroids were identified using data of ultraviolet (UV) detection and mass-spectral data as well as standard retention times. Quantification was carried out on the basis of standard calibration curves using LC Solution version 1.24 (Shimadzu, Kyoto, Japan).

RESULTS AND DISCUSSION

The *Caryophyllaceae* is a large family, with 86 genera and some 2200 species mostly distributed in the Northern Hemisphere in extra-tropical regions.[14]

Until date, approximately 323 species of 66 genera have been analyzed. No positive species have been detected in the genera: *Arenaria, Cerastium, Gypsophila, Minuartia, Spergula, Spergularia,* and *Stellaria*. Many positive species occur in other genera: *Lychnis*, *Petrocoptis* and *Silene*. Most data regarding ecdysteroid distribution are available for the *Silene*/*Lychnis* complex, into which *Melandrium* has been subsumed. Among 700 species of *Silene* of the world flora, 160 has been analyzed and include 96 species containing ecdysteroids, 52 species lacking them and there are controversial data for 12 species. The genus *Lychnis* numbers about 89 species in the world flora, 24 species of those have been tested for the presence of ecdysteroids, of which 18 are positive, 5 are negative and 1 is uncertain.[15]

2, 3 and their 2-deoxy-derivatives are the most frequently present in the *Caryophyllaceae*,[3,16] 2 is usually a predominant component, and the other phytoecdysteroids are in minor amounts.

The experimental data on ecdysteroid distribution in the plant world will possibly bring scholars closer to solving an important ecological problem - clarifying the functions of ecdysteroids in plants as well as allowing us to reveal possible uses of this group of compounds in plant chemotaxonomy.

In this investigation, we have carried out screening of the East Asian representatives of the *Caryophyllaceae* for 1, 2, 3, and 4 contents [Figure 1].

We have improved the described recently method of simultaneous identification of these four ecdysteroids[17] that belong to the same metabolic branch.[18]

Standards of each compound, as well as their mixture, were studied by HPLC with UV sequence and mass-selective detection. We improved conditions for HPLC analysis of the mixture of the four compounds and found that reliable information on ecdysteroid content can be obtained in a short time [13 min, Figure 2].

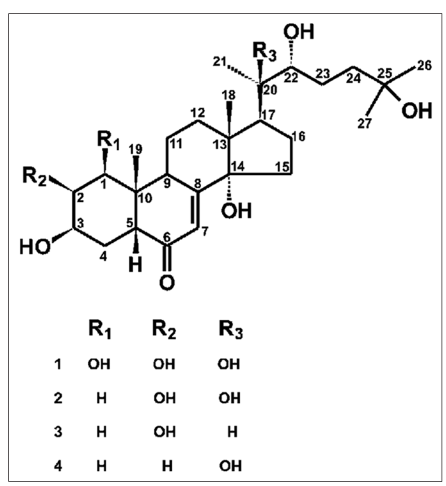

Figure 1: Chemical structures of ecdysteroids 1-4

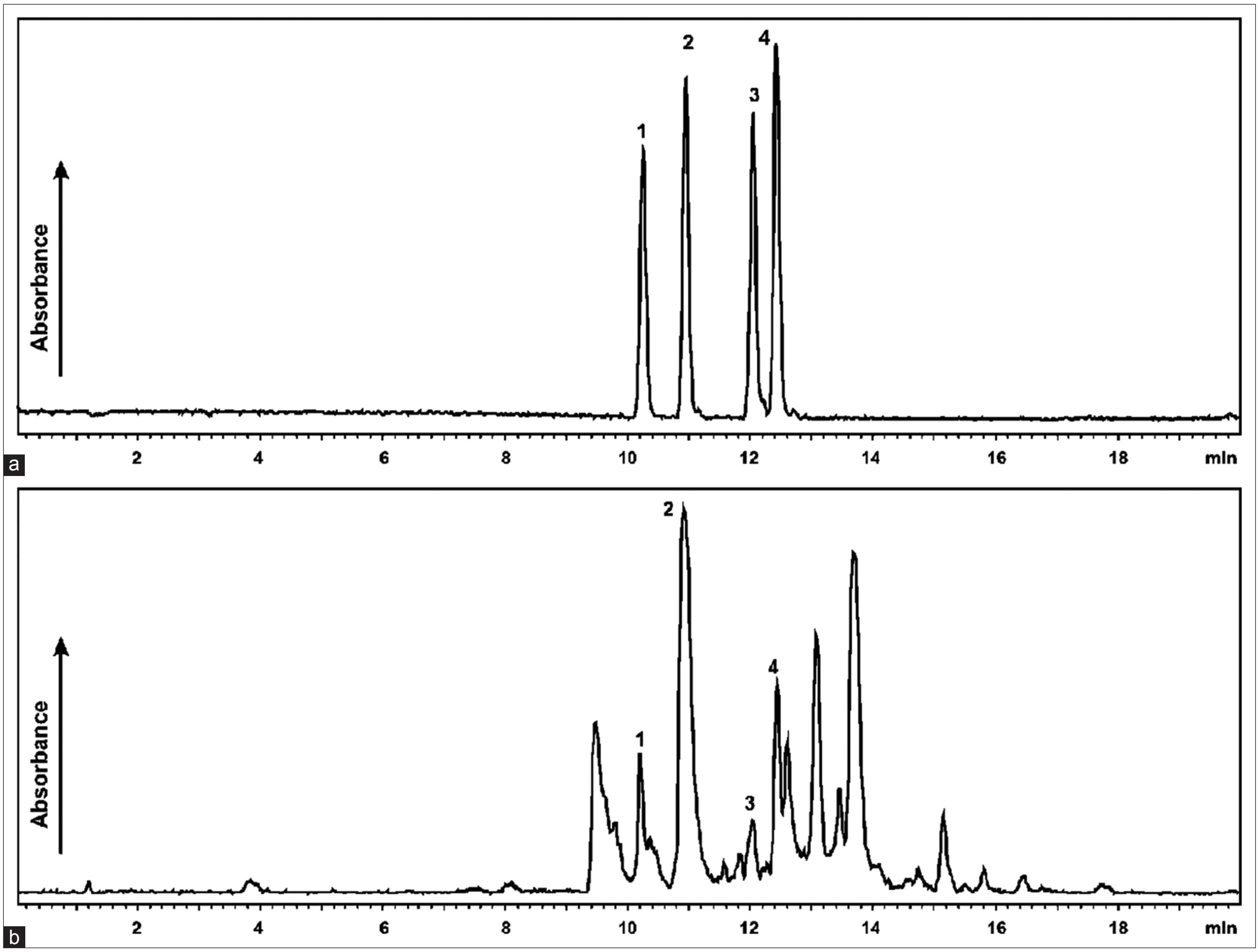

Figure 2: High-perfomance liquid chromatography profile of ecdysteroid standard mixture (a); detection: UV 246 nm. Integristerone A (1), 20-hydroxyecdysone (2), ecdysone (3) and 2-deoxy-20-hydroxyecdysone (4) and extract from the aerial part Lychnis fulgens (b)

While applying standards of the individual ecdysteroids, their elution sequence from nonpolar sorbents was determined as 1 → 2 → 3 → 4 [Figure 2a]. The last two compounds are isomers in their hydroxyl group location, which leads to their good resolvability in the chromatogram. The structure differences of the investigated ecdysteroids [Figure 1] result in some similarities and differences in their mass-spectral patterns. The intensity of the quasi-molecular ions of 2 and 4 comprised 74 and 100%, respectively, and their fragmentation was similar. Contrary to this finding, in the mass spectra of 1 and 3, the intensity of the quasi-molecular ions was 4.5 and 10%, respectively. This can be explained by the instability of the polyhydroxylated intermediates produced by APCI, with a rapid loss of water.[19,20]

Mass-spectral pattern of 1-4 coincided with data described recently.[21,17] The using a high-resolution mass-spectrometry allowed to determine the composition of all fragment ions of each investigated components [Table 1].

For extensive screening, it was necessary to apply a sensitive and specific method. We have been employed HPLC for study on the presence or absence of phytoecdysteriods in 64 species (21 genera), which are allocated to 3 subfamilies: *Paronychioideae, Alsinoideae* and *Caryophylloideae.* The results of the research on the East Asian ecdysteroids positive *Caryophyllaceae* are summarized in Table 2.

Spergularia rubra (L.) J. et C. Presl., (the subfamily *Paronychioideae*, the tribe *Sperguleae* Vierh.) has turned out to be ecdysteroid-negative.

In the subfamily *Alsinoideae* Vierh. no ecdysteroids have been detected in *Scleranthus annuus* L. (the tribe *Sclerantheae* Vierh.) and in representatives of the tribe *Alsineae* Pax: *Arenaria redowskii* Cham. et Schlecht., *Cerastium holosteoides* Fries, *Cerastium pauciflorum* Stev. ex Ser., *Cerastium fischerianum* Ser., *Cerastium beeringianum* Cham. et Schlecht., *Cerastium arvense* L., *Eremogone juncea* (Bieb.) Fenzl, *Eremogone capillaris* (Poir.) Fenzl, *Eremogone* *tschuktschorum* (Regel) Ikonn., *Fimbripetalum radians* (L.) Ikonn., *Honckenya oblongifolia* Torr. et Gray, *Minuartia arctica* (Stev. ex Ser.) Graebn., *Minuartia macrocarpa* (Pursh) Ostenf., *Minuartia biflora* (L.) Schinz et Thell., *Minuartia verna* (L.) Hiern, *Minuartia stricta* (Sw.) Hiern, *Minuartia sibirica* (Regel et Til.) N.S. Pavlova, *Moehringia lateriflora* (L.) Fenzl, *Pseudostellaria sylvatica* (Maxim.) Pax, *Pseudostellaria rigida* (Kom.) Pax, *Stellaria bungeana* Fenzl, *Stellaria graminea* L., *Stellaria media* (L.) Vill., *Stellaria eschscholtziana* Fenzl, *Stellaria fischeriana* Ser., *Stellaria ruscifolia* Pall. ex Schlecht., *Stellaria discolor* Turcz., *Stellaria humifusa* Rottb., *Stellaria cherleriae* (Fisch. ex Ser.) F. Williams, *Stellaria longifolia* Muehl. ex Willd., *Stellaria uliginosa* Murr., *Stellaria calycantha* (Ledeb.) Bong., *Stellaria filicaulis Makino* and *Stellaria dichotoma* L.

In the subfamily *Caryophylloideae,* ecdysteroids have not been revealed in the species of the tribe *Diantheae* Pax: *Dianthus chinensis* L., *Dianthus repens* Willd., *Dianthus barbatus* L., *Dianthus superbus* L., *Gypsophila pacifica* Kom., *Gypsophila violacea* (Ledeb.) Fenzl, *Gypsophila patrinii* Ser., *Gypsophila davurica* Turcz. ex Fenzl, *Psammophiliella muralis* (L.) Ikonn. As well as in the species of the tribe *Lychnideae* A. Br.: *Oberna behen* (L.) Ikonn., *Saponaria officinalis* L., *Melandrium album* (Mill.) Garcke, *Melandrium olgae* Maxim., *Melandrium apricum* (Turcz. ex Fisch. et Mey.) Rohrb., *Agrostemma githago* L., *Lychnis ajanensis* (Regel et Til.) Regel and *Silene acaulis* (L.) Jacq.

Screening the plants growing in the East Asian have confirmed the data on the absence of ecdysteroids in the species of *Arenaria, Cerastium, Gypsophila, Honckenia, Minuartia, Scleranthus, Spergularia, Agrostemma* published previously by other researchers.[11-13,15]

In a number of genera ecdysteroid-negative as well as ecdysteroid-positive species. Ecdysteroids are detected in *Dianthus deltoides* and *Saponaria bellidifolia*,[13] but no ecdysteroids have been found in the East Asian representatives of *Dianthus* and *Saponaria*, which shows their patchy distribution within the genus.

We have not detected any ecdysteroids in *O. behen*, which confirms some other researchers' data[11,12] on the lack of ecdysteroids in this species; nevertheless, ecdysteroids have been revealed in the individuals of this species growing in the European part of the Russian Federation.[22]

Ecdysteroid-containing species have been detected in the subfamilies *Alsinoideae* and *Caryophylloideae*. However, among 34 studied species of 9 genera of the tribe *Alsineae* of the subfamily *Alsinoideae*, there is only one ecdysteroid-containing representative-*Sagina maxima* A. Gray. Ecdysteroids 1, 3, and 4 have not been found [Table 2].

Ecdysteroids have been detected in 10 species of the genera *Lychnis, Silene* and *Melandrium* of the tribe *Lychnideae*, the subfamily *Caryophylloideae* [Table 2].

Lychnis is represented with five species in the Russian Far East. We analyzed all five species of which four species have turned out to be ecdysteroid-containing ones: *Lychnis wilfordii* (Regel) Maxim., *Lychnis cognata* Maxim., *Lychnis fulgens* Fisch. ex Curt. and *Lychnis sibirica* L. In *Lychnis ajanensis* (Regel et Til.) Regel ecdysteroids have not been revealed. This species was described as *Melandrium biflorum β. ajanense* Regel et Til.[23] Later Regel and Tiling raised taxon to the species.[24] V. N. Voroshilov transferred *L. ajanensis* to *Silene* genus (*Silene ajanensis* (Regel et Tiling) Worosh).[25]

In all four species the predominant compound is 2.

It is the 1st time that 1 has been found in *L. cognata* and *L. fulgens,* 3 in *L. cognata,* 4 in *L. fulgens.*

Table 1: Chromatographic (HPLC) and spectral (UV, MS) characteristics of ecdysteroids 1-4

Compounds	Rt (min)*	λ_{max}, nm (lg ε)	Ion, m/z (relative intensity, %)*
1	10.07	246 (4.09)	$[M+H]^+$, 497.3064 (4.5); $[M+H-H_2O]^+$, 479.2936 (22); $[M+H-2H_2O]^+$, 461.2841 (100); $[M+H-3H_2O]^+$, 443.2774 (31); $[M+H-4H_2O]^+$, 425.2676 (4); $[M+H-5H_2O]^+$, 407.2499 (0.5); $[M+H-C_4H_{10}O-2H_2O]^+$, 387.2130 (21); $[M+H-C_6H_{14}O_2]^+$, 379.2118 (2.5); $[M+H-C4H10O-3H2O]^+$, 369.1982 (0.9); $[M+H-C_6H_{14}O_3]^+$, 363.2136 (9.5); $[M+H-C_6H_{14}O_3-H_2O]^+$, 345.2010 (5); $[M+H-C_6H_{14}O_3-2H_2O]^+$, 327.1915 (1.8); $[M+H-C_8H_{16}O_3-H_2O]^+$, 319.1881 (17); $[M+H-C_8H_{18}O_3-H_2O]^+$, 317.1706 (1.5)
2	10.92	246 (4.23)	$[M+H]^+$, 481.3091 (74); $[M+H-H_2O]^+$, 463.2995 (32); $[M+H-2H_2O]^+$, 445.2909 (100); $[M+H-3H_2O]^+$, 427.2823 (31); $[M+H-4H_2O]^+$, 409.2713 (4); $[M+H-5H_2O]^+$, 391.2564 (1); $[M+H-C4H10O-2H2O]^+$, 371.2187 (15); $[M+H-C_6H_{14}O_3]^+$, 347.2185 (19.5); $[M+H-C_6H_{14}O_3-H_2O]^+$, 329.208 (6); $[M+H-C_8H_{16}O_3-H_2O]^+$, 303.1942 (10); $[M+H-C_8H_{18}O_3-H_2O]^+$, 301.1794 (2)
3	12.04	246 (4.12)	$[M+H]^+$, 465.3154 (10); $[M+H-H_2O]^+$, 447.3049 (100); $[M+H-2H_2O]^+$, 429.2994 (60); $[M+H-3H_2O]^+$, 411.2887 (3.5); $[M+H-4H_2O]^+$, 393.2769 (0.1); $[M+H-C_6H_{14}O_3]^+$, 331.2239 (10.5)
4	12.42	246 (4.09)	$[M+H]^+$, 465.3146 (100); $[M+H-H_2O]^+$, 447.3049 (30.5); $[M+H-2H_2O]^+$, 429.2994 (80); $[M+H-3H_2O]^+$, 411.2887 (24); $[M+H-4H_2O]^+$, 393.2769 (2); $[M+H-C_4H_{10}O-2H_2O]^+$, 355.2231 (23); $[M+H-C_6H_{14}O_2]^+$, 347.2203 (2); $[M+H-C_6H_{14}O_3]^+$, 331.2237 (11.5); $[M+H-C_6H_{14}O_3]^+$, 329.2088 (2); $[M+H-C_6H_{14}O_3-H_2O]^+$, 313.2137 (5.5); $[M+H-C_8H_{16}O_3-H_2O]^+$, 287.1985 (12); $[M+H-C_8H_{18}O_3-H_2O]^+$, 285.1824 (2)

*UV signal. HPLC: High-performance liquid chromatography; UV: Ultraviolet

Table 2: Contents of integristerone A (1), 20-hydroxyecdysone (2), ecdysone (3) and 2-deoxy-20-hydroxyecdysone (4) in the *Caryophyllaceae* aerial parts

Subfamilies/ tribes/species	Contents (μg/mg dry weight) of ecdysteroids			
Alsinoideae/ Alsineae	1	2	3	4
//Sagina maxima	ND	1.03±0.05	ND	ND
Caryophylloideae/ Lychnideae				
//Lychnis wilfordii	0.12±0.01	1.52±0.07	ND	ND
//Lychnis cognata	0.011±0.001*	0.75±0.04	0.12±0.01*	ND
//Lychnis fulgens	0.011±0.001*	1.15±0.06	0.12±0.01	0.10±0.01*
//Lychnis sibirica	ND	0.30±0.01	ND	ND
//Silene repens	0.24±0.01*	0.84±0.02	ND	ND
//Silene foliosa	0.08±0.01*	3.85±0.24	ND	ND
//Silene stenophylla	0.50±0.02*	4.76±0.20	ND	0.50±0.02*
//Silene jenisseensis	0.012±0.001*	1.60±0.08	ND	0.17±0.01*
//Melandrium sachalinense	0.67±0.03*	2.30±0.10*	ND	ND
//M. firmum	ND	0.11±0.01*	ND	ND

ND: Not detected (<0.01). *Identified for the 1st time

The previous data[13,26] on the presence of 1, 2 in *L. wilfordii* and 2 in *Lychnis cognate* have been confirmed.

Earlier 2, 3 and polypodine B were identified in the Far Eastern *L. fulgens*.[27] The results of our research have also detected 2 and 3 in this taxon.

The genus *Silene* is represented with nine species in the Russian Far East. We have investigated five species, of which 4 have turned out to be ecdysteroid-containing: *Silene foliosa, Silene stenophylla, Silene jenisseensis* (the section *Chloranthae* (Rohrb.) Schischk.) and *Silene repens* (the section *Spergulifoliae*). In *S. acaulis* (the section *Nanosilene* Otth.), ecdysteroids have not been detected, although there is some information on the presence of ecdysteroids in this species, in a number of papers.[12,13,28]

It is the 1st time that 1 has been revealed in *S. repens* Patrin, *S. foliosa* Maxim., *S. stenophylla* Ledeb. and *S. jenisseensis* Willd., maximum content of which reaches 0.49 μg/mg in the aerial part in *S. stenophylla* during its blooming period. In the aerial part of *S. stenophylla* and *S. jenisseensis,* 3 have been detected for the 1st time.

The predominant component in all the investigated East Asian species of the genus *Silene* is 2, its content in the aerial part if the investigated species varies from 0.83 μg/mg in *S. repens* to 4.7 μg/mg in *S. stenophylla*.

The genus *Melandrium* is represented with seven species in the Russian Far East. Five species have been analyzed, of which two species turned out to be ecdysteroid-containing. It is the 1st time that 2 has been found in *Melandrium firmum* (Siebold et Zucc.) Rohrb. and *M. sachalinense* (Fr. Schmidt) Schischk., in the latter 1 has been detected as well.

CONCLUSION

Ecdysteroid-containing species have been detected in two subfamilies of the *Caryophyllaceae*: *Paronychioideae* and *Caryophylloideae*. Most species containing ecdysteroids are representatives of the tribe *Lychnideae* of the subfamily *Caryophylloideae*. In the tribe *Diantheae* (the subfamily *Caryophylloideae*), no sources of ecdysteroids have been found. It can be asserted that ecdysteroid-containing taxa are confined to the tribe *Lychnideae*, however, the distribution of ecdysteroids in the genera of the tribe *Lychnideae* is patchy. Along with genera containing ecdysteroids there are ecdysteroid-negative taxa.

The analysis of ecdysteroid content in the species of *Silene, Lychnis* and *Melandrium* revealed that a genus may include species containing ecdysteroids as well as ones in which they have not been identified. In the genus *Silene,* ecdysteroids have been revealed in the species, which belong to the sections *Chloranthae* and *Spergulifoliae*. The plants of these sections are the ones in which finding of new ecdysteroid sources can be prognosed. Data on the quantity of ecdysteroids are available as for several species of *Caryophyllaceae* of particular interest. According to our results the ecdysteroid content is 4-5 μg/mg in aerial part of *Silene* species.

Most perspective sources of ecdysteroid are species of the *Silene*. Perspective sources of ecdysteroids are species from *Silene*.

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