Phytoecdysteroids of the East Asian Caryophyllaceae

Elena Novozhilova, Viacheslav Rybin¹, Petr Gorovoy, Irina Gavrilenko, Roman Doudkin²

Laboratory of Plant Chemotaxonomy, G.B. Elyakov Pacific Institute of Bioorganic Chemistry of the Far Eastern Branch of the Russian Academy of Sciences, ¹Laboratory of Comparative Biochemistry, A.V. Zhirmunsky Institute of Marine Biology of the Far Eastern Branch of the Russian Academy of Sciences, ²Far East Federal University, Vladivostok, Russia

Submitted: 13-01-2014 Revised: 24-02-2014 Published: 27-05-2015

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 Alsideae; Scleranthus annuus of the tribe Scleranthaceae, as well as the East Asian representatives of the genera Gypsophila, Psammophiliela, Dianthus and Saponaria of the tribe Diantheae; Obena and Agrostemma of the tribe Lychnideae. Conclusion: This investigation shows the most promising sources of ecdysteroids 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]

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 C18 (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 (N2) pressure was 25 kPa. The nebulizer gas (N2) 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
While applying standards of the individual ecdysteroids, their elution sequence from nonpolar sorbents was determined as $1 \rightarrow 2 \rightarrow 3 \rightarrow 4$ [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 phytoecdysteroids 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 Sclerentheae Vierh.) and in representatives of the tribe Alsineae Pax: *Arenaria redowskii* Cham. et Schlecht., *Cerastium bolsteoides* Fries, *Cerastium paeoniflorum* Stev. ex Ser., *Cerastium fischerianum* Ser., *Cerastium beeringianum* Cham. et Schlecht., *Cerastium arvense* L., *Eremogone juncea* (Bieb.) Fenzl, *Eremogone capillaris* (Poir.) Fenzl, *Eremogone...
In the subfamily Caryophylloideae, ecdysteroids have not been revealed in the species of the tribe Diantheae Pax: \textit{Dianthus chenensis} L., \textit{Dianthus repens} Willd., \textit{Dianthus barbatus} L., \textit{Dianthus superbus} L., \textit{Gypsophila pacifica} Kom., \textit{Gypsophila violacea} (Ledeb.). Fenzl, \textit{Gypsophila patrinii} Ser., \textit{Gypsophila davurica} Turcz. ex Fenzl, \textit{Psammophylla murrallii} (L.) Ikonn. As well as in the species of the tribe \textit{Lychnideae} A. Br.: \textit{Oboeba behen} (L.) Ikonn., \textit{Saponaria officinalis} L., \textit{Melandrium album} (Mill.) Garcke, \textit{Melandrium olgae} Maxim., \textit{Melandrium apricum} (Turcz. ex Fisch. et Mey.) Rohrb., \textit{Agrostemma githago} L., \textit{Lychnis ajanensis} (Regel et Til.) Regel and \textit{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 \textit{Arenaria}, \textit{Cerastium}, \textit{Gypsophila}, \textit{Honckenia}, \textit{Minuartia}, \textit{Selenanthus}, \textit{Spergularia}, \textit{Agrostemma} published previously by other researchers. 

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

We have not detected any ecdysteroids in \textit{O. behen}, which confirms some other researchers’ data 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.

Ecdysteroid-containing species have been detected in the subfamilies \textit{Alsinioideae} and \textit{Caryophylloideae}. However, among 34 studied species of 9 genera of the tribe \textit{Alsinioideae} of the subfamily \textit{Alsinioideae}, there is only one ecdysteroid-containing representative- \textit{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 \textit{Lychnis}, \textit{Silene} and \textit{Melandrium} of the tribe \textit{Lychnideae}, the subfamily \textit{Caryophylloideae} [Table 2].

\textit{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: \textit{Lychnis wilfordii} (Regel) Maxim., \textit{Lychnis cognata} Maxim., \textit{Lychnis fulgens} Fisch. ex Curt. and \textit{Lychnis sibirica} L. In \textit{Lychnis ajanensis} (Regel et Til.) Regel ecdysteroids have not been revealed. This species was described as \textit{Melandrium biffurum} \textit{b. ajanense} Regel et Til.[23] Later Regel and Tiling raised taxon to the species.[24] V.N. Voroshilov transferred \textit{L. ajanensis} to \textit{Silene} genus (\textit{Silene ajanensis} (Regel et Tiling) Worosh.).[25]

In all four species the predominant compound is 2.

It is the 1\textsuperscript{st} time that 1 has been found in \textit{L. cognata} and \textit{L. fulgens}, 3 in \textit{L. cognata}, 4 in \textit{L. fulgens}.

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

| Compounds | Rt (min)* | $\lambda_{max}$ nm (lg ε) | Ion, m/z (relative intensity, %)* |
|-----------|-----------|-----------------|-------------------------------|
| 1         | 10.07     | 246 (4.09)      | [M+H]+, 497.3064 (4.5); [M+H+2H,O]+, 479.2936 (22); [M+H+2H,O]+, 461.2841 (100); [M+H-3H,O]-, 443.2774 (31); [M+H+4H,O]+, 425.2676 (4); [M+H+5H,O]+, 407.2499 (0.5); [M+H+C,H,O-2H,O]+, 387.2130 (21); [M+H+4H,C,H,O]-, 379.2118 (2.5); [M+H+4H,C,H,O]-, 369.1982 (0.9); [M+H+4H,C,H,O]-, 359.2049 (0.9); [M+H+4H,C,H,O]-, 349.2016 (5); [M+H+4H,C,H,O]-, 339.1981 (17); [M+H+4H,C,H,O]-, 319.1841 (17); [M+H+4H,C,H,O]-, 317.1706 (15.5). |
| 2         | 10.92     | 246 (4.23)      | [M+H]+, 481.3091 (74); [M+H+2H,O]+, 463.2955 (32); [M+H+2H,O]+, 445.2909 (100); [M+H+3H,O]+, 427.2803 (31); [M+H+4H,O]+, 409.2713 (4); [M+H+5H,O]+, 391.2664 (1); [M+H+4H,C,H,O]-, 391.2654 (1); [M+H+4H,C,H,O]-, 371.1817 (15); [M+H+4H,C,H,O]-, 347.2185 (19.5); [M+H+4H,C,H,O]-, 329.2086 (6); [M+H+4H,C,H,O]-, 303.1942 (10); [M+H+4H,C,H,O]-, 301.1794 (2). |
| 3         | 12.04     | 246 (4.12)      | [M+H]+, 465.3154 (10); [M+H+2H,O]+, 447.3049 (100); [M+H+2H,O]+, 429.2994 (60); [M+H+3H,O]+, 411.2887 (3.5); [M+H+4H,O]+, 393.2769 (0.1); [M+H+4H,C,H,O]-, 331.2239 (10.5). |
| 4         | 12.42     | 246 (4.09)      | [M+H]+, 465.3146 (100); [M+H+2H,O]+, 447.3049 (30.5); [M+H+2H,O]+, 429.2994 (80); [M+H+3H,O]+, 411.2887 (24); [M+H+4H,O]+, 393.2769 (2); [M+H+4H,C,H,O]-, 355.2231 (23); [M+H+4H,C,H,O]-, 347.2203 (2); [M+H+4H,C,H,O]-, 331.2237 (11.5); [M+H+4H,C,H,O]-, 329.2088 (2); [M+H+4H,C,H,O]-, 313.2137 (5.5); [M+H+4H,C,H,O]-, 287.1985 (12); [M+H+4H,C,H,O]-, 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        |                                            |
| //Sagina maxima             | ND                                         |
| //Caryophylloideae/Lychniodeae | 1.03±0.05*                                |
| //Lychnis wilfordii         | 0.12±0.01                                 |
| //Lychnis cognata           | 0.01±0.01*                                |
| //Lychnis fulgens           | 0.01±0.01*                                |
| //Lychnis sibirica          | ND                                         |
| //Silene repens             | 0.24±0.01*                                |
| //Silene foliosa            | 0.08±0.01*                                |
| //Silene stemophylla        | 0.50±0.02*                                |
| //Silene jenisseensis       | 0.012±0.001*                              |
| //Melandrium sachalinense    | 0.67±0.03*                                |
| //M. firmum                 | ND                                         |

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

The previous data[12,20] 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 stemophylla, Silene jenisseensis (the section Chloranthae (Rohrb.) Schischk.) and Silene repens (the section Spergulifoliae). In S. aculis (the section Nanosiene Orth.), 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. stemophylla Ledeb. and S. jenisseensis Willd., maximum content of which reaches 0.49 μg/mg in the aerial part of S. stemophylla during its blooming period. In the aerial part of S. stemophylla 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. stemophylla.

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 Dianthae (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.

REFERENCES

1. Sláma K, Koudela K, Tenora J, Mathová A. Insect hormones in vertebrates: Anabolic effects of 20-hydroxyecdysone in Japanese quail. Experientia 1996;52:702-6.
2. Todorov IN, Mitrokhin YI, Efremova OA, Sidorenko LI. Impact of extracts from Leuzea carthamoides on biosynthesis of RNA and proteins in mouse organs. Khimico-Farmatsevticheskii Zhurnal 2000;34:24-6.
3. Volodin VN, Shisharova TI, Barsyeva SA, Melnik MV. Biological activity of 20-hydroxyecdysone and its acetates. Rastitel'nye Resursy 1999;35:76-81.
4. Abubakirov NK. Ecdysteroids of the flowering plants (Angiospermae). Khimiya Prirodnykh Soedinenii 1981;6:685-702.
5. Baltava AE, Rashkei YA, Darmogray VN, Belov YP, Abubakirov NK. Phytoecdysteroids of the Silene nutans. II. 22-Deoxyecdysterone and its mass spectral characteristics. Khimiya Prirodnykh Soedinenii 1985;1:62-6.
6. Baltava AE, Darmogray VN, Abubakirov NK. Phytoecdysteroids of plants of the genus Silene. XIV. Ecdysterone 20-O-benzoate
from Silene tatarica. Khimiya Prirodnykh Soedinenii 1987;6:850-2.
7. Saatov Z, Usmanov BZ, Abubakirov NK. Phytoecdysones of Silene praemixta. I. Silenosterone. Khimiya Prirodnykh Soedinenii 1979;6:793-7.
8. Saatov Z, Gorovits MB, Abdullah ND, Usmanov BZ, Abubakirov NK. Phytoecdysteroids from Silene plants. VIII. 2-Deoxyecdysterone 3-acetate from Silene praemixta. Khimiya Prirodnykh Soedinenii 1985;1:60-2.
9. Saatov Z, Gorovits MB, Abubakirov NK. Phytoecdysteroids in plants of the genus Silene. Khimiya Prirodnykh Soedinenii 1993;5:627-35.
10. Lafont R, Horn DH. Phytoecdysteroids: Structures and occurrence. In: Koolman J, editor. Ecdysone: From Chemistry to Mode of Action. Stuttgart: Georg Thieme Verlag; 1989. p. 39-64.
11. Revina TA, Revushkin AS, Rakitin AV. Ecdysteroid-containing species in flora of the Altai mountains. Rastitel'nye Resursy 1988;34:565-70.
12. Volodin V, Chadin I, Whiting P, Dinan L. Screening plants of European North-East Russia for ecdysteroids. Biochem Syst Ecol 2002;30:525-78.
13. Zibareva LN, Dinan L, Eryomina VI. Screening of Caryophyllaceae species for phytoecdysteroids presence. Rastitel'nye Resursy 2007;43:66-75.
14. Brummitt RK. Vascular Plant Families and Genera. Kew: Royal Botanic Gardens; 1992. p. 804.
15. Zibareva L, Volodin V, Saatov Z, Savchenko T, Whiting P, Lafont R, et al. Distribution of phytoecdysteroids in the Caryophyllaceae. Phytochemistry 2003;64:499-517.
16. Bergamasco R, Horn DH. Distribution and role insect hormones in plants. In: Downer RG, Laufer H, editors. Endocrinology of Insects. New York: A.R. Liss; 1983. p. 627-57.
17. Rybin V, Boltenkov E, Novozhilova E. Application of high-performance liquid chromatography for simultaneous identification of integristerone A, 20-hydroxyecdysone, ecdysone and 2-deoxy-20-hydroxyecdysone. Nat Prod Commun 2007;2:1101-4.
18. Rees HH, Mendis AH. The occurrence and possible significance of ecdysteroids during nematode and cestode development. In: Hoffmann JA, Porchot P, editors. Biosynthesis, Metabolism and Mode of Action of Invertebrate Hormones. Berlin: Springer-Verlag; 1984. p. 338-45.
19. Stevens JF, Reed RL, Morré JT. Characterization of phytoecdysteroid glycosides in Meadowfoam (Limnanthes alba) seed meal by positive and negative ion LC-MS/MS. J Agric Food Chem 2008;56:3945-52.
20. Wainwright G, Prescott MC, Lomas LO, Webster SG, Rees HH. Trace analysis of arthropod hormones by liquid chromatography-atmospheric pressure chemical ionization/ mass spectrometry. Biochem Soc Trans 1996;24:476.
21. Destrez B, Pinel G, Bichon E, Monteauf L, Le Bizec B. Detection of 20-hydroxyecdysone in calf urine by comparative liquid chromatography/high-resolution mass spectrometry and liquid chromatography/tandem mass spectrometry measurements: Application to the control of the potential misuse of ecdysteroids in cattle. Rapid Commun Mass Spectrum 2008;22:4073-80.
22. Darmogray VN, Serova TG. Phytochemical studies on the plants of the genus Obara Adams. of the family Caryophyllaceae Juss. Sovremennye naukoyomkie tekhnologii 2004;5:111-2.
23. Regel E, Tiling SH. Florula Ajanenesis. Nouveaux Memoires de la Soc. Nat. de Moscou 1859;11:1-128.
24. Regel E. Aufzählung der von Radde in Baikalien, Dahurien und am Amur gesammelten Pflanzen. Bull Nat Moscou 1861;34:458-578.
25. Voroshilov VN. Key of the Plants of Soviet Far East. Moscow: Nauka; 1982. p. 253.
26. Zibareva LN, Baltaev UA, Sviridova TP, Saatov Z, Abubakirov NK. Species of the genus Lychnis L. - Potential sources of ecdysteroids. Rastitel'nye Resursy 1995;31:1-9.
27. Baltaev UA, Gorovoi PG, Abubakirov NK. Phytoecdysteroids of Lychnis fulgens. Khimiya Prirodnykh Soedinenii 1986;6:794-5.
28. Zibareva L. Distribution and levels of phytoecdysteroids in plants of the genus Silene during development. Arch Insect Biochem Physiol 2000;43:1-8.

Cite this article as: Novozhilova E, Rybin V, Gorovoy P, Gavrilenko I, Doudkin R. Phytoecdysteroids of the East Asian Caryophyllaceae. Phcog Mag 2015;11:225-30.

Source of Support: Nil, Conflict of Interest: None declared.