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Structure and Stereochemistry of Phytoecdysone from *Silene Cretaceae Fisch*

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Abstract—2-Deoxyecdyzone (3β,14α,22R,25-tetraoxy-5β-cholest-7-en-6-one) has been isolated from Silene cretaceae Fisch. Caryophyllaceae. The compound structure has been elucidated by X-ray diffraction studies.

Keywords: phytoecdysone, *Silene cretaceae Fisch.*, X-ray diffraction

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Research on the structure and stereochemistry of α-ecdysone, including positions and configuration of oxygen-containing substituents, was quite recent. The results have served as a basis for elucidation of structure of other related compounds isolated from plants, phytoecdysones and phytoecdysteroids.

Phytoecdysteroids are known as widely spread secondary vegetable metabolites: more than 150 of their structures have been identified. Phytoecdysteroids exhibit anabolic, hypoglycemic, hepatoprotective, adaptogenic, and restorative action. Moreover, they may prevent atherosclerosis and are used for treatment of myocarditis and bone fractures [1]. In modern medicine, compounds of this class are used as anabolic [2], adaptogenic, and restorative drugs as well as biostimulants [3].

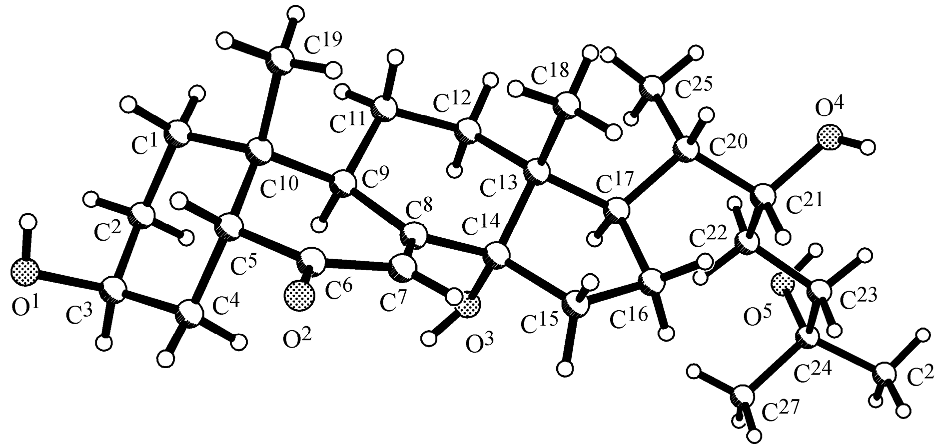
Even though the content of ecdysteroids vary significantly even in the case of closely related species, it has been confirmed that certain families and geniuses include more ecdysteroids-rich plants [4] and are therefore attractive for screening of promising ecdysteroids.

Studies of Kazakhstan plants with respect to ecdysteroids have been scarce. The *Caryophyllaceae Juss*. and *Asteraceae Dumort.,* families populating Central Kazakhstan are the most interesting in this regard [5]. For example, phytochemical and pharmaceutical studies have resulted in development of the first Kazakh ecdysteroid drug, "Ekdifit," showing anabolic, adaptogenic, and restorative activities. This drug is produced from *Serratula coronata L. Asteraceae Dumort*. containing 1.5% of ecdysteron in the raw material (HPLC) [6].

We have studied 28 plant species of the *Caryophyllaceae Juss.* family. Relatively high content of the above-mentioned ecdysteroid was found in the cases of *Saponaria calabrica* (0.1%), *Lychnis chalcedonica* (0.45%), *Gypsophila rupestris* (0.14%), *Silene altaica* (0.14%), and *Silene cretaceae Fisch.* (0.51%). The most striking finding was that *Silene wolgensis* (*Hornem*) *Bess*. contained 1.76% of ecdysteron, more than did *Serratula coronata L.*; therefore, the former species can be potentially used as the major source of 20-hydroxyecdysone [7–10].

Containing a variety of basic and minor ecdysteroids, the *Silene* genius (the *Caryophyllaceae* family) plants are attractive as well [11–15]. In view of this, we studied the ecdysteroid composition of the top of *Silene cretaceae Fisch.* belonging to the above-mentioned family. Reverse-phase HPLC study revealed the ecdysteron contents of 0.51 in its ethanolic extract. Along with ecdysteron, minor 2-deoxyecdysone **I** was isolated.

X-ray diffraction study of the parent ecdysteron (20-hydroxyecdysone) was reported in 1971, followed



General view of 2-deoxyecdysone (**I**) molecule.

by X-ray diffraction analysis of structures of several ecdysteroid derivatives [16]. However, data on crystal and molecular structure of 2-deoxyecdysone were lacking; therefore, in this work we report the first X-ray diffraction study of 2-deoxyecdysone (see its general view in the figure).

From the collected data it followed that the bond lengths and bond angles were close to their ordinary values (Tables 1 and 2) [17]. The six-membered ring $C^1C^2C^3C^4C^5C^{10}$ (A) was in the almost ideal *chair* conformation ($\Delta C_S^3 = 0.7°$). The $C^5C^6C^7C^8C^9C^{10}$ ring (B) existed as a significantly flattened distorted *semichair* ($\Delta C_2^{7,8} = 6.2°$), and the $C^8C^9C^{11}C^{12}C^{13}C^{14}$ ring (C) was in the form of distorted *chair* ($\Delta C_S^9 = 3.6°$). The five-membered ring $C^{13}C^{14}C^{15}C^{16}C^{17}$ (D) was found to be in the shape of distorted 13α,14β-*semichair* ($\Delta C_2^{13,14} = 5.3°$) (the transannular torsion angles are compiled in Table 3). Similar conformations of the A–D rings were previously reported for the other ecdysteroids [18–20]. The junction of rings A and B was of the *cis* type, common of other ecdysteroids (the $H^5C^5C^{10}C^{19}$ torsion angle was 49.2°); that of B and C rings was of the *pseudotrans* type ($\angle C^7C^8C^9C^{10} = 10.7°$ and $\angle C^{14}C^8C^7C^{11} = -43.9°$); whereas rings C and D were in the *trans* junction ($\angle O^3C^{14}C^{13}C^{18} = 175.7°$). The hydroxy group at C^3 was in equatorial position, and the carbohydrate substituent at C^{17} was in pseudoequatorial orientation.

In crystal, the molecules formed infinite ribbons along the 2_1 crystallographic axis (0, *y*, 0) due to the formation of hydrogen bonds O^1–H (*x*, *y*, *z*)···O^2 (–*x*, 0.5 + *y*, 1/2 – *z*) (O^1···O^2 2.77 Å, H···O^2 1.99 Å, ∠O–H···O 158.7°), O^3–H (1 – *x*, 0.5 + *y*, 0.5 – *z*)···O^1 (*x*, *y*, *z*) (O^2···O^1 2.76 Å, H···O^1 1.94 Å, ∠O–H···O 173.8°), O^4–H (*x*, *y*, *z*)···O^5 (–1 + *x*, *y*, *z*) (O^4···O^5 2.75 Å, H···O^5 2.03 Å, ∠O–H···O 170.7°), and O^4–H (*x*, *y*, *z*)···O^5 (–0.5 + *x*, 0.5 – *y*, 1 – *z*) (O^4···O^5 2.74 Å, H···O^5 1.91 Å, ∠O–H···O 177.5°).

EXPERIMENTAL

The crystal cell parameters and intensity of 4822 independent reflections were measured with the Bruker APEX-II CCD diffractometer (MoK_α, graphite monochromator, θ/2θ scan at 2θ < 51°). The crystals were rhombic, the crystal cell parameters were as follows: *a* = 6.610(3) Å, *b* = 9.894(4) Å, *c* = 41.548(17) Å,

Table 1. Bond lengths (*d*, Å) in molecule of **I**

Bond	*d*	Bond	*d*
O^1–C^3	1.439(3)	C^{10}–C^{19}	1.537(4)
O^2–C^6	1.231(3)	C^{11}–C^{12}	1.528(4)
O^3–C^{14}	1.441(4)	C^{12}–C^{13}	1.529(4)
O^4–C^{21}	1.439(4)	C^{13}–C^{18}	1.541(4)
O^5–C^{24}	1.443(4)	C^{13}–C^{14}	1.543(4)
C^1–C^2	1.513(4)	C^{13}–C^{17}	1.555(4)
C^1–C^{10}	1.541(4)	C^{14}–C^{15}	1.510(4)
C^2–C^3	1.513(4)	C^{15}–C^{16}	1.546(4)
C^3–C^4	1.509(4)	C^{16}–C^{17}	1.553(4)
C^4–C^5	1.539(4)	C^{17}–C^{20}	1.534(4)
C^5–C^6	1.505(4)	C^{20}–C^{25}	1.535(5)
C^5–C^{10}	1.546(4)	C^{20}–C^{21}	1.536(5)
C^6–C^7	1.445(4)	C^{21}–C^{22}	1.529(5)
C^7–C^8	1.339(4)	C^{22}–C^{23}	1.517(5)
C^8–C^{14}	1.511(4)	C^{23}–C^{24}	1.532(5)
C^8–C^9	1.512(4)	C^{24}–C^{27}	1.518(5)
C^9–C^{11}	1.547(4)	C^{24}–C^{26}	1.527(5)
C^9–C^{10}	1.570(4)		

Table 2. Bond angles (ω, deg) in molecule of **I**

Angle	ω	Angle	ω
$C^{2}C^{1}C^{10}$	114.4(2)	$C^{18}C^{13}C^{14}$	109.9(2)
$C^{3}C^{2}C^{1}$	112.8(2)	$C^{12}C^{13}C^{17}$	117.5(2)
$O^{1}C^{3}C^{4}$	111.2(2)	$C^{18}C^{13}C^{17}$	110.3(3)
$O^{1}C^{3}C^{2}$	112.8(2)	$C^{14}C^{13}C^{17}$	100.9(2)
$C^{4}C^{3}C^{2}$	111.5(3)	$O^{3}C^{14}C^{15}$	108.5(3)
$C^{3}C^{4}C^{5}$	113.0(2)	$O^{3}C^{14}C^{8}$	107.5(2)
$C^{6}C^{5}C^{4}$	106.0(2)	$C^{15}C^{14}C^{8}$	118.9(2)
$C^{6}C^{5}C^{10}$	112.5(2)	$O^{3}C^{14}C^{13}$	105.2(2)
$C^{4}C^{5}C^{10}$	113.4(2)	$C^{15}C^{14}C^{13}$	103.8(2)
$O^{2}C^{6}C^{7}$	121.2(3)	$C^{8}C^{14}C^{13}$	112.2(2)
$O^{2}C^{6}C^{5}$	121.1(3)	$C^{14}C^{15}C^{16}$	104.3(3)
$C^{7}C^{6}C^{5}$	117.3(2)	$C^{15}C^{16}C^{17}$	107.0(2)
$C^{8}C^{7}C^{6}$	123.0(3)	$C^{20}C^{17}C^{16}$	113.3(3)
$C^{7}C^{8}C^{14}$	122.4(3)	$C^{20}C^{17}C^{13}$	118.3(3)
$C^{7}C^{8}C^{9}$	123.1(3)	$C^{16}C^{17}C^{13}$	103.1(2)
$C^{14}C^{8}C^{9}$	114.4(2)	$C^{17}C^{20}C^{25}$	112.7(3)
$C^{8}C^{9}C^{11}$	111.9(2)	$C^{17}C^{20}C^{21}$	112.4(3)
$C^{8}C^{9}C^{10}$	113.4(2)	$C^{25}C^{20}C^{21}$	111.5(3)
$C^{11}C^{9}C^{10}$	112.8(2)	$O^{4}C^{21}C^{22}$	108.5(3)
$C^{19}C^{10}C^{1}$	108.3(2)	$O^{4}C^{21}C^{20}$	108.9(3)
$C^{19}C^{10}C^{5}$	107.7(2)	$C^{22}C^{21}C^{20}$	115.7(3)
$C^{1}C^{10}C^{5}$	107.4(2)	$C^{23}C^{22}C^{21}$	112.6(3)
$C^{19}C^{10}C^{9}$	111.5(2)	$C^{22}C^{23}C^{24}$	116.7(3)
$C^{1}C^{10}C^{9}$	111.0(2)	$O^{5}C^{24}C^{27}$	105.1(3)
$C^{5}C^{10}C^{9}$	110.7(2)	$O^{5}C^{24}C^{26}$	109.4(3)
$C^{12}C^{11}C^{9}$	115.8(3)	$C^{27}C^{24}C^{26}$	110.9(3)
$C^{11}C^{12}C^{13}$	111.7(2)	$O^{5}C^{24}C^{23}$	109.3(3)
$C^{12}C^{13}C^{18}$	110.3(3)	$C^{27}C^{24}C^{23}$	112.2(3)
$C^{12}C^{13}C^{14}$	107.3(2)	$C^{26}C^{24}C^{23}$	109.8(3)

Table 3. Torsion angles (τ, deg) in molecule of **I**

Angle	τ	Angle	τ
Ring A		Ring C	
$C^{10}C^{1}C^{2}C^{3}$	54.7(3)	$C^{9}C^{8}C^{14}C^{13}$	55.5(3)
$C^{1}C^{2}C^{3}C^{4}$	−51.2(3)	$C^{12}C^{13}C^{14}C^{8}$	−60.8(3)
$C^{2}C^{3}C^{4}C^{5}$	51.0(3)	$C^{11}C^{12}C^{13}C^{14}$	57.6(4)
$C^{3}C^{4}C^{5}C^{10}$	−53.9(3)	$C^{9}C^{11}C^{12}C^{13}$	−50.5(4)
$C^{4}C^{5}C^{10}C^{1}$	52.9(3)	$C^{8}C^{9}C^{11}C^{12}$	41.9(4)
$C^{2}C^{1}C^{10}C^{5}$	−53.8(3)	$C^{14}C^{8}C^{9}C^{11}$	−43.9(3)
Ring B		Ring D	
$C^{10}C^{5}C^{6}C^{7}$	−36.9(3)	$C^{13}C^{14}C^{15}C^{16}$	−35.2(3)
$C^{5}C^{6}C^{7}C^{8}$	7.2(4)	$C^{14}C^{15}C^{16}C^{17}$	10.7(4)
$C^{6}C^{7}C^{8}C^{9}$	6.4(4)	$C^{15}C^{16}C^{17}C^{13}$	17.5(4)
$C^{7}C^{8}C^{9}C^{10}$	10.7(4)	$C^{14}C^{13}C^{17}C^{16}$	−38.2(3)
$C^{8}C^{9}C^{10}C^{5}$	−38.9(3)	$C^{17}C^{13}C^{14}C^{15}$	46.1(3)
$C^{6}C^{5}C^{10}C^{9}$	51.8(3)		

$V = 2717.2(2)$ Å^3, $Z = 4$, $C_{27}H_{44}O_5$, $d_{calc} = 1.170$ g/cm^3, space group $P2_12_12_1$. The structure was solved by the direct method. The positions and thermal parameters of nonhydrogen atoms were refined under anisotropic approximation by the full-matrix least-squares method. Hydrogen atoms were placed in positions calculated from geometry considerations and were included into the refinement by the *rider* model, except for hydrogen atoms of hydroxy groups at O^4 and O^5 that were found from the differential synthesis of electron density. The calculation accounted for 3011 reflections with $I > 2\sigma(I)$. The final divergence factors were $R = 0.0489$ and $wR_2 = 0.1021$. The structure was solved applying SHELXS-97 and SHELXL-97 software [21]. The structural data were deposited in Cambridge Crystallographic Data Center (CCDC 828330).

Isolation of 2-deoxyecdysone from *Silene cretaceae Fisch.* The raw material was harvested at rocky mountainside (Mudzhara, Aktyubinsk region, Kazakhstan) during blossoming season.

1 kg of chopped air-dried raw material was extracted fourfold with 70% aqueous ethanol (boiling, water bath). The so obtained ethanol extract (350 g) was treated with 450 mL of mixture of petroleum ether and ethyl acetate (2 : 1) in order to remove the nonpolar components, the residue was extracted with isobutanol to get thick extract. The isobutanol extracts were combined, and isobutanol was evaporated to dryness in a vacuum. The so obtained syrup (165 g) was separated by HPLC [eluents: chloroform–ethanol (30 : 1) (fractions 1–18) and chloroform–ethanol (5 : 1) (fractions 19–42)]. Fractions 1–18 containing 2-deoxyecdysone **I** were recrystallized from ethyl acetate. From fractions 19–42, 1.41 g of ecdysteron and 0.564 g of 2-deoxyecdysone were isolated. 2-Deoxyecdysone: purity 98.2%, mp 207°C (ethyl acetate – methanol), $[\alpha]_D^{20}$ 66.0 (1.0, MeOH). IR spectrum (KBr), ν, cm^{-1}: 3320, 2950, 1650, 1450,

1200, 1080, 880. UV spectrum (EtOH), λ_{max}, nm: 244 (log ε 4.10).

REFERENCES

1. Yatsuk, Ya.K. and Segal', G.M., *Khim. Prirod. Soedin.*, 1970, no. 1, p. 28.
2. Zibareva, L.N., *Doctoral (Chem.) Dissertation*, Novosibirsk, 2003.
3. Saatov, Z., Syrov, V.N., Mamatkhanov, A.U., and Abubakirov, N.K., *Khim. Prirod. Soedin.*, 1994, no. 2, p. 152.
4. Baltaev, U.A., *Bioorgan. Khim.*, 2000, vol. 26, no. 12, p. 892.
5. Dinan, L., *Eur. J. Entomol.*, 1995, vol. 92, p. 271.
6. Tuleuov, B.I., *Steroidnye soedineniya rastenii i lekarstvennye preparaty na ikh osnove. Poisk, khimicheskaya modifikatsiya i prakticheskie aspekty primeneniya* (Steroid Compounds and Plant Drugs on Them. Search, Chemical Modification, and Practical Applications), Karaganda: Glasir, 2009, p. 208.
7. Krasikov, V.D., *Osnovy planarnoi khromatografii* (Basics of Planar Chromatography), St. Petersburg: Khimizdat, 2005, p. 232.
8. Tuleuov, B.I., Abstracts of Papers, *XVIII Mendeleevskii s''ezd po obshchei i prikladnoi khimii* (Mendeleev Congr. on General and Applied Chemistry), Moscow, 2007, p. 472.
9. Habdolda, G., Ivasenko, S.A., Bek, S.A., Sorokin, R.N., Smagulova, F.M., Tuleuov, B.I., Baltaev, U.A., and Adekenov, S.M., Abstracts of Papers, *7 Int. Symp. on the Chemistry of Natural Compounds*, Tashkent, 2007, p. 288.
10. Tuleuov, B.I., Abstracts of Papers, *V Vseros. nauch. konf. "Khimiya i tekhnologiya rastitel'nyh veshchestv"* (V Al-Russ. Conf. "Chemistry and Technology of Plant Compounds"), Ufa, 2008, p. 294.
11. *Flora SSSR*, Moscow: Akad. Nauk SSSR, 1936, vol. 6, p. 647.
12. *Flora Kazakhstana,* Alma-Ata: Akad. Nauk KazSSR, 1960, vol. 3, p. 378.
13. Saatov, Z., Usmanov, B.Z., and Abubakirov, N.K., *Khim. Prirod. Soedin.*, 1979, no. 6, p. 793.
14. Zibareva, L.N., Dainen, L., and Eremina, V.I., *Rast. Resursy*, 2007, vol. 43, no. 4, p. 66.
15. Ramazonov, N.Sh., Mamadalieva, N.Z., and Bobaev, I.D., *Khim. Prirod. Soedin.*, 2007, no. 1, p. 97.
16. Akhrem, A.A. and Kovganko, N.V., *Ekdisteroidy: Khimiya i biologicheskaya aktivnost'* (Ecdysteroids: Chemistry and Biological Activity), Moscow: Nauka i Tekhnika, 1989, p. 327.
17. Allen, F.H., Kennard, O., Watson, D.G., Brammer, L., Orpen, A.G., and Taylor, R., *J. Chem. Soc. Perkin Trans. 2*, 1987, p. 1.
18. Camps, F., Coll, J., Cortel, A., Molins, E., and Miravittes, C., *J. Chem. Res.*, 1985, vol. 14, p. 241.
19. Fabian, L., Argay, G., Kalman, A., and Bathori, M., *Acta Cryst. (B)*, 2002, vol. 58, p. 710.
20. Okuzumi, K., Hara, N., Uekusa, H., Fujimoto, Y., *Org. Biomol. Chem.*, 2005, vol. 3, p. 1227.
21. Sheldrick, G.M., *SHELXL-97. Program for the Refinement of Crystal Structure*, University of Göttingen, Germany, 1997.