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PHYTOECDYSTEROIDS FROM THE *Silene* GENUS

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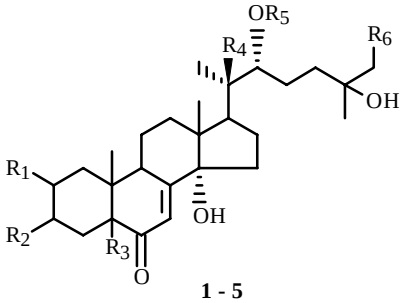
Research results on the isolation and identification of ecdysteroids from plants of the Silene *L. genus were presented.*

Key words: phytoecdysteroids, distribution, isolation, *Silene* genus.

The genus *Silene* L. is a good source of new ecdysteroid analogs [1-5]. Species of this genus contain 59% of ecdysteroids. Ecdysteroids have been found by us in 115 species of *Silene* including in 75 species for the first time [6]. Of these, 26 species had a high content of them. Until now the chemical composition of these compounds has been determined in 23 *Silene* species including 12 investigated by us.

We established for the first time the ecdysteroid profile of the following species: *Silene antirrhina, S. chlolirifolia, S. cretica, S. disticha, S. echinata, S. italica, S. linicola, s. portensis, S. pseudotites, S. radicosa, S. regia, S. viridiflora* [4, 5, 7] using a developed detection method in seeds [8]. These data were subsequently confirmed by radioimmunoassay (Table 1). The studied species possess agonistic (ecdysteroid) activity but not one of them exhibited antagonistic activity. This confirms that they contain phytoecdysteroids.



1 - 5

1: $R_1 = R_3 = R_5 = H$, $R_2 = R_4 = R_6 = OH$
2: $R_1 = R_3 = R_4 = R_6 = H$, $R_2 = OH$, $R_5 = \beta$-D-Glc
3: $R_1 = R_5 = R_6 = H$, $R_2 = \beta$-D-Glc, $R_3 = R_4 = OH$
4: $R_1 = R_4 = R_6 = OH$, $R_2 = OAc$, $R_3 = H$, $R_5 = Ac$
5: $R_1 = OAc$, $R_2 = R_4 = R_6 = OH$, $R_3 = H$, $R_5 = OAc$

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TABLE 1. Analysis of Caryophyllaceae Plant Extracts for Ecdysteroid Content

Plant species	Growth phase	Plant part	CSP analysis	RIA DBL-1	Biotest for agonists
S. antirrhina	Fr	Fruit,	0.5	969	+++
		seeds	0.1	87.2	++-
S. echinata	Fl	Flowers,	0.4	5972	+++-
	Fr	seeds	0.1	101.4	++-
S. viridiflora	B	Buds,	1.6	6582	++++-
	Fr	seeds	0.2	659	+++--
S. frivaldszkyana	Fl	Flowers,	6.2	27592	++++-
	Fr	seeds	0.6	113.2	+++--
S. portensis	Fl	Flowers,	1.4	15312	C+++-
	Fr	seeds	0.2	86.2	++-
S. linicola	Fl	A. part,	0.5	2000	+++-
	Fr	seeds	0.6	1485	+++(+)-
S. radicosa	Fl	Flowers	1.3	11393	++++-
S. pseudotites	E	Leaves	0.6	27188	+++-
S. italica	Fl	Flowers	1.3	12205	++++-
S. cretica	Fr	Fruit,	0.4	1920	+++-
		seeds	0.3	49.1	++-
S. disticha	Fl	Leaves,	0.4	2555	C++-
	Fr	seeds	0.3	109.5	++-
S. chlorifolia	E	Leaves	0.8	3020	+++-

B, budding; Fl, flowering; Fr, fruiting; E, end of vegetation; A. part, aerial part. CSP, chromatospectrophotometric method, % of dry material. RIA, radioimmunoassay, µg ecdysone equivalent/g. C, cytotoxic; -, inactive; +, undiluted extract active; (+), weakly active; ++, +++, ++++, active at 10-, 100-, and 1000-fold, respectively, dilution.

The main ecdysteroid components were identified in seven species (*S. antirrhina*, *S. chlorifolia*, *S. cretica*, *S. disticha*, *S. echinata*, *S. italica*, *S. portensis*) by HPLC using the UV spectra of the peaks. The absorption maximum is due to the presence of a conjugated ketone and occurs near 242-250 nm. Semipreparative HPLC over a C_{18} column was used to concentrate the pure compounds. The retention times of the resulting peaks compared with those of polypodine B (P_B), 20-hydroxyecdysone (20E), 25*R*-inocosterone, 25*S*-inocosterone, 2-deoxy-20-hydroxyecdysone, and ecdysone and co-injection with the proposed ecdysteroid standards enabled the compounds to be identified on two analytical reversed-phase C_{18} and C_6 columns (Table 2). The major components in each *Silene* species are 20E and P_B. Ecdysteroids **1**-**5** were isolated for the first time. Glycosides are typically found for the *Silene* genus. For example, the series of glycosides sileneoside A, B, C, D, and E was found in *S. brachuica* [9-13], *S. nutans*, *S. scabrifolia*, *S. supina*, *S. viridiflora* [14, 15]; in *S. otites*, 20E 25-glycoside [3]. However, 2-deoxyecdysone 22β-D-glycoside and 2-deoxypolipodine B 3-O-β-D-glycoside were isolated for the first time. The presence in plants of the ecdysteroid glycosides indicates that they play an active role in metabolic processes [1].

EXPERIMENTAL

Extracts were purified using Sep-Pac Vac 35-cc cartridges. A Gilson Model 811 HPLC chromatograph combined with a Gilson 160 detector and the Gilson Unipoint computer program were used for analysis. HPLC separation of the extracts was performed over Spherisorb 5 ODS-2 analytical columns with C_{18} and C_6 (particle size 5 mm, 150 × 4.6 mm, Jones Chromatography).

TABLE 2. Phytoecdysteroids Isolated from *Silene* L. Species

Ecdysteroid	Formula	Yield, %	Plant species
20-Hydroxyecdysone	$C_{27}H_{44}O_7$	0.071	*S. pseudotites*
		0.367	*S. linicola*
		0.042	*S. radicosa*
		0.302	*S. regia*
		0.350	*S. viridiflora*
Polipodine B	$C_{27}H_{44}O_8$	0.006	*S. pseudotites*
		0.073	*S. linicola*
		0.007	*S. radicosa*
		0.021	*S. regia*
		0.250	*S. viridiflora*
25*S*-Inocosterone	$C_{27}H_{44}O_7$	Tr.	*S. pseudotites*
		0.012	*S. regia*
Ecdysone	$C_{27}H_{44}O_6$	0.002	*S. pseudotites*
		0.001	*S. linicola*
2-Deoxyecdysone	$C_{27}H_{44}O_5$	0.165	*S. pseudotites*
2-Deoxy-20-hydroxyecdysone	$C_{27}H_{44}O_6$	0.106	*S. pseudotites*
		0.017	*S. linicola*
		0.200	*S. viridiflora*
Ponasterone A	$C_{27}H_{44}O_6$	Tr.	**
Sidisterone	$C_{24}H_{32}O_6$	0.001	*S. pseudotites*
2-Deoxyintegristerone A	$C_{27}H_{44}O_7$	0.001	*S. pseudotites*
(5α-)-2-Deoxyintegristerone A	$C_{27}H_{44}O_7$	0.001	*S. pseudotites*
2-Deoxy-21-hydroxyecdysone	$C_{27}H_{44}O_6$	0.013	*S. pseudotites*
Viticosterone E	$C_{29}H_{46}O_8$	0.001	*S. linicola*
Turkesterone	$C_{27}H_{44}O_8$	0.001	*S. linicola*
Integristerone A	$C_{27}H_{44}O_8$	0.002	*S. linicola*
		0.200	*S. viridiflora*
Sileneoside A	$C_{33}H_{54}O_{12}$	0.080	*S. viridiflora*
Sileneoside D	$C_{33}H_{54}O_{12}$	0.100	*S. viridiflora*
26-Hydroxypolipodine B	$C_{27}H_{44}O_9$	0.035	*S. viridiflora*
2-Deoxy-20,26-dihydroxyecdysone (**1**)*	$C_{27}H_{44}O_7$	0.003	*S. pseudotites*
2-Deoxyecdysone 22β-D-glycoside (**2**)*	$C_{33}H_{54}O_{10}$	0.003	*S. pseudotites*
2-Deoxypolipodine B 3β-D-glycoside (**3**)*	$C_{33}H_{54}O_{12}$	0.003	*S. pseudotites*
20,26-Dihydroxyecdysone 3,22-diacetate (**4**)*	$C_{31}H_{48}O_{10}$		*S. viridiflora*
20,26-Dihydroxyecdysone 2,22-diacetate (**5**)*	$C_{31}H_{48}O_{10}$		*S. viridiflora*

*Ecdysteroids isolated for the first time, **detected in most species; Tr.: traces.

Radioimmunoassay and biotest B_{II} for the presence and determination of ecsdysteroid content in the plants were carried out at Exeter University (Exeter, Great Britain). IR spectra were recorded on a Perkin—Elmer 2000 FT-spectrometer. NMR spectra were recorded on JNM-4H-100, Bruker WP 200 SY, Bruker Avance DRX400, and Bruker AMX500 instruments at 27°C using standard Bruker microprograms. The solvents were C_5D_5N, CD_3OD, and D_2O with hexamethyldisiloxane (HMDS), tetramethylsilane (TMS), and sodium [2,2,3,3-$^2H_4$]-3-(trimethylsilyl)propionate internal standards.

TABLE 3. ^{1}H and ^{13}C NMR Chemical Shifts of 20,26-Dihydroxyecdysone 3,22-Diacetate (**4**) and 20,26-Dihydroxyecdysone 2,22-Diacetate (**5**) (D_2O, δ, ppm, J/Hz)

C atom	^{13}C (4)	^{1}H (4)	^{13}C (5)	^{1}H (5)
1	37.7	1.48, 1.99	37.7	1.53, 1.96
2	-	4.13 (m, $w_{1/2}$ = 22)	-	5.08 (m, $w_{1/2}$ = 22)
3	72.3	5.17 (m, $w_{1/2}$ = 8)	73.0	4.22 (m, $w_{1/2}$ = 8)
4	-	1.78, 1.85	-	1.80, 1.83
5	51.9	2.35 (dd, J = 4.2, 13.5)	51.5	2.41 (dd, 5.13)
6	-	-	-	-
7	122.4	5.98 (d, J = 2.5)	122.3	5.99 (d, J = 2.5)
8	-	-	-	-
9	35.0	3.11 (m, $w_{1/2}$ = 24)	35.1	3.18 (m, $w_{1/2}$ = 24)
10	39.1	-	39.5	-
11	-	1.74, 1.86	-	1.73, 1.86
12	32.0	1.76, 1.96	32.3	1.76, 1.96
13	48.5	-	48.7	-
14	86.3	-	86.6	-
15	31.3	1.67, 2.06	31.6	1.67, 2.06
16	20.7	1.88, 1.76	21.4	1.88, 1.76
17	50.6	2.31 (t, J = 9.5)	50.6	2.31 (t, J = 9.5)
18	18.1	0.86 s	18.2	0.86 s
19	24.2	1.02 s	24.1	1.02 s
20	78.4	-	78.4	-
21	21.3	1.34 s	21.5	1.34 s
22	82.7	4.85 (dd, J = 10.5, 2)	81.4	4.85 (dd, J = 10.5, 2)
23	-	1.56, 1.76	-	1.56, 1.76
24	35.9	1.75, 1.46	35.9	1.75, 1.46
25	74.4	-	74.5	-
26	69.9	3.42 s	69.8	3.42 s
27	23.2	1.15 s	23.1	1.15 s
2-CH_3CO	-	-	22.1/175.0	2.127 s
3-CH_3CO	21.7/175.1	2.176 s	-	-
22-CH_3CO	21.7/176.0	2.165 s	21.9/176.1	2.167 s

-, not determined

Mass spectra were recorded on Finnigan MAT-8200 and JEOL JMS-700 (desorption-chemical ionization) mass spectrometers using ammonia as a reagent. Certain mass spectra were obtained on a triple quadrupole (Quattro triple quadrupole) VG mass spectrometer with liquid secondary-ion ionization (LSIMS) using a Cs^+ beam and a glycerine matrix.

2-Deoxy-20,26-dihydroxyecdysone (1), $C_{27}H_{44}O_7$, M.W. 480, UV spectrum (EtOH, λ_{max}, nm, log ε): 242 (4.01).

HPLC: t_R 23.7 [Zorbax-Sil, CH_2Cl_2:$(CH_3)_2CHOH$:H_2O, 125:40:3]. Mass spectrum (CI, NH_3) (*m*/*z*, I_{rel}, %): 498 (81) $[M + H + NH_3]^+$, 481 (54) $[M + H]^+$, 480 (100) $[M]^+$, 463 (10.5) $[M + H - H_2O]^+$, 445 (4) $[M + H - 2H_2O]^+$.

PMR spectrum (D_2O, δ, ppm, J/Hz): 0.86 (3H, s, C_{18}-Me), 0.98 (3H, br.s, $w_{1/2}$ = 4, C_{19}-Me), 1.16 (3H, s, C_{27}-Me), 1.24 (3H, s, C_{21}-Me), 2.33 (t, J = 9.7, H-17), 2.39 (br.dd, J = 12.5, J = 3.4, H-5), 3.15 (br.s, $w_{1/2}$ = 25, H-9), 3.44 (d, J = 10.5, H-22), 3.45 [s, CH_2OH(26)], 5.96 (d, H-7, J = 2.2), 4.10 (br.s, $w_{1/2}$ = 20, H-3).

2-Deoxyecdysone 22*β*-D-glycoside (2), $C_{33}H_{54}O_{10}$, M.W. 610, HPLC: t_R 23.5 [Zorbax-Sil, CH_2Cl_2:$(CH_3)_2CHOH$:H_2O, 125:40:3].

Mass spectrum (CI, NH_3) (*m*/*z*, I_{rel}, %): 628 (100) $[M + H + NH_3]^+$, 611 (18) $[M + H]^+$, 610 (41) $[M]^+$, 593 (7) $[M + H - H_2O]^+$.

PMR spectrum (D_2O, δ, ppm, J/Hz): 0.74 (3H, s, C_{18}-Me), 0.956 (3H, d, J = 6.8, C_{21}-Me), 0.990 (3H, br.s, $w_{1/2}$ = 4, C_{19}-Me), 1.238 (3H, s, C_{26}-Me), 1.243 (3H, s, C_{27}-Me), 2.18 (m, H-17), 2.41 (dd, J = 12.3, J = 3.2, H-5), 3.14 (br.m, $w_{1/2}$ = 25, H-9), 3.73 (br.d, J = 10.5, H-22), 4.10 (br.s, $w_{1/2}$ = 23, H-3), 5.97 (d, J = 2.1, H-7), 4.53 (d, J = 8, H-1′), 3.29 (dd, J = 9.1, J = 8.1, H-2′), 3.48 (t, J = 9.3, H-3′), 3.38 (t, J = 9.3, H-4′), 3.45 (m, H-5′), 3.70 (dd, J = 12.4, J = 6.2, H-6′), 3.89 (dd, J = 12.3, J = 2.3, H-6′).

^{13}C NMR (C_5D_5N, δ, ppm): 101.8 (C-1).

2-Deoxypolipodine B 3β-D-glycoside (3), $C_{33}H_{54}O_{12}$, M.W. 642. HPLC: t_R 38.0 [Zorbax-Sil, CH_2Cl_2:$(CH_3)_2CHOH$:H_2O, 125:40:3].

Mass spectrum (CI, NH_3) (*m*/*z*, I_{rel}, %): 480 (9) $[M - sugar]^+$, 463 (40) $[M + H - sugar - H_2O]^+$, 180 (46) $[sugar]^+$.

PMR spectrum (D_2O, δ, ppm, J/Hz): 0.875 (3H, s, C_{18}-Me), 0.905 (3H, s, C_{19}-Me), 1.221 (3H, s, C_{26}-Me), 1.233 (3H, s, C_{21}-Me), 1.233 (3H, s, C_{27}-Me), 3.44 (d, J = 10.5, H-22), 2.32 (t, J = 9.3, H-17), 3.243 (m, H-9), 4.31 (br.s, $w_{1/2}$ = 12, H-3), 5.99 (d, J = 2.1, H-7), 4.56 (d, J = 8, H-1′), 3.30 (dd, J = 9, J = 8, H-2′), 3.51 (t, J = 9.3, H-3′), 3.39 (t, J = 9.3, H-4′), 3.45 (m, H-5′), 3.71 (dd, J = 12.4, J = 5.9, H-6′), 3.90 (dd, J = 12.3, J = 2, H-6′).

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