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ECDYSTEROSIDE, A PHYTOECDYSTEROID FROM *SILENE TATARICA*

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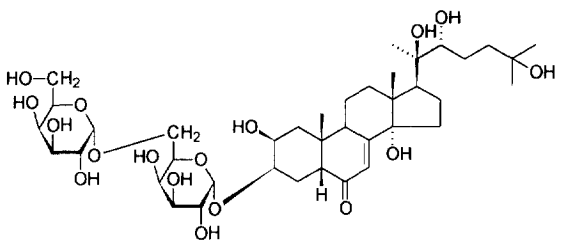
Key Word Index—*Silene tatarica*; Caryophyllaceae; ecdysteroid; ecdysteroside; 20-hydroxyecdysone 3-[*O*-α-D-galactopyranosyl(1-6)]-*O*-α-D-galactopyranoside.

Abstract—A new ecdysteroid glycoside, ecdysteroside, was isolated from an extract of whole plants of *Silene tatarica* (L.) Pers. Using physico-chemical methods it was identified as the 3-[*O*-α-D-galactopyranosyl(1-6)]-*O*-α-D-galactopyranoside of 20-hydroxyecdysone.

INTRODUCTION

While conducting a study on phytoecdysteroids of the plant *Silene tatarica* (L.) Pers., we isolated two phytoecdysteroids of glycoside character containing mono- (**1**) and digalactose (**2**) residues, repectively, in the sugar portion from a middlepolar fraction. Based on physico-chemical and spectral data, the first compound was identified as the 3-*O*-α-D-galactopyranoside of 20-hydroxyecdysone [1], and the diglycoside was found to be a new compound and named ecdysteroside (**2**).



2

RESULTS AND DISCUSSION

The mass-spectrum of compound **2** did not reveal a molecular peak. The ions with *m/z* 588 (3%), 573 (3%), 570 (6%), 555 (2.1%), 552 (2.4%), 534 (2.1%), 516 (1.5%), 501, 462, 455, 444 (30%), 426 (36%), 411 (15%), 408 (15%), 393 (10%), 375, and 357 characterize the high mass region of the mass spectrum. Fragment ions with *m/z* 345 (12%) and 327 were formed on cleavage of the C-20/C-22 bond. An ion with *m/z* 300 (100%) corresponded to the breaking of the C-17/C-20 bond. The fragments of the steroid part of compound **2** described above are analogous to the mass-spectral fragmentation of the steroid part of 20-hydroxyecdysone. The mass spectra of ecdysteroids with a side-chain analogous to that of 20-hydroxyecdysone on the cleavage of the C-20/C-22 bond lead to side-chain fragments with *m/z* 99 and, after dehydration, *m/z* 81. Ions with *m/z* 99 (30%) and 81 (27%) were present in the mass spectrum of **2**, which indicated that the side-chain of ecdysteroside (**2**) and that of 20-hydroxyecdysone are identical. With the help of the ^{1}H and ^{13}C NMR spectra and using 2D correlation spectroscopy (2D COSY), the positions of the substituents on the steroid rings and side-chain of **2** have been determined. In the ^{13}C NMR spectra of 3-*O*-α-D-galactopyranoside of 20-hydroxyecdysone [1] and ecdysteroside (**2**), the signals of the carbinol hydrogen atoms C-2, C-3, C-14, C-20 and C-25 were evident. Since the signals for C-3 in **1** and **2** are more deshielded than C-3 in 20-hydroxyecdysone, glycosylation was present at C-3 in **2**. In the ^{13}C NMR spectrum of ecdysteroid **2** (Table 1), the signals for the carbon atoms of the sugar residues have chemical shifts as follows: δ 103.71 (C-1′); 71.02 (C-2′); 71.78 (C-3′); 71.02 (C-4′); 73.46 (C-5′); 62.62 (C-6′); 101.75 (C-1″); 70.95 (C-2″); 71.66 (C-3″); 70.56 (C-4″); 72.59 (C-5″) and 62.62 (C-6″). According to ref. [2], these data indicate a pyranose conformation of D-galactose. In the ^{13}C NMR spectrum of D-galactofuranose the chemical shifts of the carbon atoms are shifted downfield, particularly that of C-4 [2, 3], when compared with those of the pyranose form. In the ^{1}H NMR spectrum of **2** (Table 2), the anomeric protons resonate at δ 5.6 with $J = 4$ Hz (C-1′) and δ 5.16 with $J = 4$ Hz (C-1″). These factors and differences in the

Table 1. ^{13}C NMR chemical shifts of ecdysteroside (**2**) (δ, ppm)

No. atom	Chemical Shift	No. atom	Chemical shift	No. atom	Chemical shift
1	38.53	14	84.12	27	30.06
2	68.08	15	31.68	C-1′	103.71
3	79.15	16	21.46	C-2′	71.02
4	31.41	17	50.10	C-3′	71.78
5	52.43	18	17.84	C-4′	71.02
6	202.97	19	24.27	C-5′	73.46
7	121.51	20	76.87	C-6′	62.62
8	166.51	21	21.67	C-1″	101.75
9	34.25	22	77.57	C-2″	70.95
10	39.39	23	22.45	C-3″	71.66
11	21.04	24	42.60	C-4″	70.56
12	31.68	25	69.57	C-5″	72.59
13	48.06	26	29.99	C-6″	62.62

Table 2. ^1H NMR chemical shifts of 20-hydroxyecdysone galactopyranoside (**1**) and ecdysteroside (**2**) (δ, ppm)

Compound	Proton signals									
	H-2	H-3	H-7	H-9	H-22	CH_3-18	CH_3-19	CH_3-21	CH_3-26/27	H-1′, H-1″
1	4.04	4.07		3.46	3.86	1.19	0.97	1.57	1.35	5.75 *d*; ^3J = 4 Hz
2	4.04	4.08	6.19	3.45	3.84	1.19	0.98	1.57	1.35	5.56*d*; 5.16 *d* ^3J = 4 Hz; ^3J = 4 Hz

molecular rotations [4] between 20-hydroxyecdysone, 3-*O*-α-D-galactopyranoside of 20-hydroxyecdysone and ecdysteroside (**2**) indicated α-configurations for the glycoside anomeric centres and that the sugar units were connected by a 1-6 bond. The sugar anomeric protons were examined by ^1H NMR methods. Double resonance was applied to a signal resonating at δ 5.56 which was found to interact with the proton signal at δ 4.62, and that interacts with a signal at δ 4.56; the signal at δ 4.28 interacts with signals at δ 4.56 and 4.38. The NOE method was applied to a signal at δ 5.16, and this was found to interact with a proton signal resonating at δ 4.60. With the help of the double resonance method the signal at δ 4.60 was found to interact with a signal resonating at δ 4.49; this method was also used to determine proton signals resonating at δ 4.49, 4.63, 4.59, and 4.39. Thus, ecdysteroside was shown to be the 3-[*O*-α-D-galactopyranosyl(1-6)]-*O*-α-D-galactopyranoside of 20-hydroxyecdysone.

EXPERIMENTAL

The mass spectra were recorded on an MX - 1310 spectrometer supplied with a direct sample inlet system into the ion source with an ionizing current of 40 V, the collector current at 50 mA, and the temperatures of the evaporating ampule and ionization chamber were 160–200°. PMR and ^{13}C NMR spectra were obtained on a Bruker AM-400 spectrometer: δ-scale, 0 = TMS.

Ecdysteroside (**2**). The combined fractions collected in the course of the study on ecdysteroids from 1.2 kg of whole plants of *Silene tatarica* were chromatographed on a column of silica gel eluted with $CHCl_3$-MeOH (4:1). Repeated chromatography in this system yielded 12 mg ecdysteroside. Mp 228–230° (EtOAc—MeOH), $[\alpha]_D^{20}$ + 100,4 (*c* 2.59; MeOH). ^1H NMR spectrum (pyridine-d_5, 400 MHz, ppm): δ 1.19 (3H, *s*, H-1), 0.98 (3H ,*s*, H-19), 1.35 (6H-26, 27), 1.57 (3H, *s*, H-21), 3.84 (1H, *dd*. H-22), 4.04 (1H, *c*, H-2), 4.08 (1H , H-3), 3.45 (1H, *m*, H-9), 5.16 (1H, *d*, ^3J = 4 Hz, H-1″), 5.56 (1H, *d*, ^3J = 4 Hz, H-1′).

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REFERENCES

1. Saatov, Z., Abdullaev, N. D., Gorovits, M. B. and Abubakirov, N. K., *Khimiya Prirod. Soedin.*, 1981, 741.
2. Beier, R. C. and Mundey, B. P., *Can. J. Chem.*, 1980, **58**, 280.
3. Shashkov, A. S. and Chizhov, O. S., *Bioorganichskaya Khimiya*, 1976, **2**, 437.
4. Klyne, W., *Biochem. J*, 1950, **61**, 47.