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PHYTOECDYSTEROIDS OF PLANTS OF THE GENUS *Silene*.

VI. α-ECDYSONE 22-SULFATE — A NEW ECDYSTEROID FROM *Silene brahuica*

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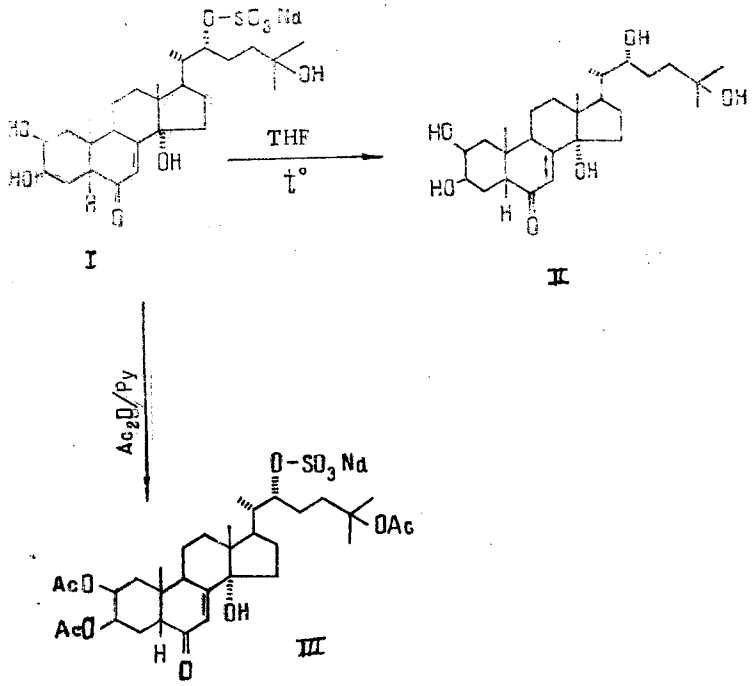
UDC 547.926:591.147

A phytoecdysteroid consisting of the sodium salt of α-ecdysone 22-sulfate has been isolated from the roots of *Silene brahuica* Boiss.

Continuing a study of the ecdysteroids of *Silene brahuica* Boiss. (family Caryophyllaceae) [1], we have isolated a new ecdysteroid (I) from the roots of this plant.



The IR spectrum of compound (I) showed, in addition to the maximum at 1652 cm^{-1} that is characteristic for ecdysteroids, absorption at 1235 cm^{-1} corresponding to a sulfate group [2]. The solvolytic cleavage of ecdysteroid (I) in tetrahydrofuran [3] gave a compound (II) identified as α-ecdysone. The SO_4^{2-} ion was detected in the reaction products by the test with $BaCl_2$. Consequently, ecdysteroid (I) contains a sulfate group.

Institute of the Chemistry of Plant Substances, Academy of Sciences of the UzbekSSR, Tashkent. Translated from Khimiya Prirodnykh Soedinenii, No. 4, pp. 467-470, July-August, 1984. Original article submitted July 4, 1983.

0009-3130/84/2004-0441$08.50

TABLE 1. Chemical Shifts (δ, ppm; C_5D_5N; 0 — TMS) and Spin–Spin Coupling Constants of α-Ecdysone 22-Sulfate (I), its Acetyl Derivative (III), and α-Ecdysone (II)

Compound	Position of the protons					
	H-2	H-3	H-5	H-7	H-9	H-20
I	4,15 dt ^3J = 12,3 4,4 and 3,5 Hz	4,23 br.s $W_{1/2}$ = 9 Hz	3,00 q ^3J = 13.2 and 4,4 Hz	6.20 d ^4J = 2,4 Hz	3,54m	3,09 m Σ^3J = 21,4 Hz
II	4,10 m	4,22 br.s	2,99 q J^3 = 13 and 4 Hz	6,18 br.s	3,52m	—
III	5,28 dt ^3J = 12,3; 4,4 and 3,5 Hz	5,49 br.s $W_{1/2}$ = 9 Hz	2,63 q ^3J = 13,0 and 3,9 Hz	6,16 d ^4J = 2,4 Hz	3,53m	3,03 m Σ^3J = 21,4 Hz

Compound	Position of the protons					
	H-22	CH_3-18	CH_3-19	CH_3-21	CH_3-26/27	OAc
I	5,21 m Σ^3J = 15,1 Hz	0,79 s	1,01 s	1,27 d ^3J=7,0 Hz	1,31 s 1,32 s	—
II	4,10 m	0,74 s	1,07 s	1,24 d	1,37 s 1,37 s	—
III	5,08 m Σ^3J = 15,6 Hz	0,78 s	1,02 s	1,28 d ^3J=6,9 Hz	1,42 s 1,42 s	1,92 s 2,01 s 2,06 s

dt — doublet of triplets; br.s — broadened singlet; m — multiplet; q — quartet; d — doublet; s — singlet.

It was shown by double resonance that the one-proton signal appearing in the PMR spectrum of compound (I) in the form of a doublet of triplets with ^3J = 12.3, 4.4, and 3.5 Hz at 4.15 ppm related the H-2 proton. It is obvious that a broadened singlet with $W_{1/2}$ = 9 Hz at 4.23 ppm must be assigned to H-3.

It can be seen from Table 1 that the magnitudes of the chemical shifts of the H-3 and H-2 atoms of the ecdysteroid (I) and of α-ecdysone (II) agreed well. The signals of the methyl groups and of the H-5, H-7, and H-9 protons had close characteristics.

In the PMR spectrum of the ecdysteroid (I) a one-proton octet with Σ^3J = 21.4 Hz was observed at 3.09 ppm. Under double-resonance conditions, with the superposition of a saturating frequency on the doublet at 1.27 ppm (CH_3-21) this signal was converted into a quartet with ^3J = 11.5 and 3.1 Hz. Such a transition showed that the multiplet under consideration related to the H-20 proton.

At 5.21 ppm there was another one-proton signal appearing in the form of a doublet of triplets with Σ^3J = 15.1 Hz. This signal simplified to a quartet with ^3J = 9.0 and 3.0 Hz on saturation of the resonance transition of the H-20 nucleus (3.09 ppm). Thus, the multiplet at 5.21 ppm must be assigned to the H-22 proton.

The signal of the H-22 proton underwent a considerable paramagnetic shift ($\Delta\delta$ = 1.11 ppm) on passing from α-ecdysone (4.10 ppm) to α-ecdysone 22-sulfate (5.21 ppm). These results indicated that the sulfate group was attached to the hydroxy group at C-22.

The acetylation of ecdysteroid (I) with acetic anhydride in pyridine at room temperature gave the 2,3,5-triacetate (III). This was shown by the presence of three three-proton signals at 1.92, 2.01, and 2.06 ppm, and also by the paramagnetic shifts of the signals of the H-2 and H-3 protons and of the 26/27 methyl groups (see Table 1). In the triacetate (III) the sulfate group was retained at C-22.

Analysis of the ^{13}C NMR spectra of ecdysteroid (I) and of α-ecdysone (II) [4, 5] also led us to the conclusion that the sulfuric acid esterified the hydroxy group at C-22 (Table 2).

TABLE 2. Chemical Shifts (ppm) of the Carbon Atoms of α-Ecdysone 22-Sulfate (I) and of α-Ecdysone (II) [5] in C_5D_5N Relative to TMS

C atom	Compound		C atom	Compound	
	I	II		I	II
1	37,8	38,3	15	32,2	32,2
2	68,1	68,3	16	26,5	26,9
3	68,1	68,3	17	48,2	48,6
4	32,0	32,5	18	17,9	16,1
5	51,2	51,5	19	24,1	24,7
6	203,5	203,7	20	35,7	42,2
7	121,5	121,6	21	19,8	15,1
8	165,6	165,8	22	82,6	74,1
9	34,5	34,9	23	24,0	25,9
10	38,6	38,9	24	41,1	42,5
11	21,7	21,4	25	69,8	70,2
12	32,1	34,1	26	31,2	30,7 b
13	47,6	47,7	27	29,5	30,7 b
14	85,3	84,2			

a, b — the assignments of signals with similar superscripts may be interchanged.

It was not difficult to observe that the values of the chemical shifts of the carbon atoms of the steroic moieties of compounds (I) and (II) were fully comparable. Appreciable changes were detectable only in the positions of the signals of the carbon atoms of the side chains.

Thus, as compared with the results for α-ecdysone (II), the C-22 signal of α-ecdysone 22-sulfate (II) was displaced downfield by +8.5 ppm, while the signals of the C-20, C-23, and C-24 carbon atoms underwent diamagnetic shifts of −3.5, −1.9, and −1.4 ppm, respectively. These changes, undoubtedly initiated by the location of a sulfate group at C-22, are in harmony with literature information [6, 7].

We isolated the sulfuric-acid-esterified ecdysteroid in the form of the sodium salt, but it is not excluded that in the plant it is present in the form of the free acid.

EXPERIMENTAL

PMR spectra were taken on a SC-500 (Varian) instrument (C_5D_5N, 0 — TMS), and ^{13}C NMR spectra on a CFT-20 instrument (Varian) (C_5D_5N, 0 — TMS); for other information, see [8].

α-Ecdysone 22-Sulfate (Sodium Salt) (I). The mother liquors collected in the accumulation of the sileneoside B [1] obtained from 10 g *Silene brahuica* were subjected to repeated column chromatography on SiO_2 in the chloroform—methanol—water (80:32:0.3) system. After the recrystallization of the appropriate fractions from methanol, 100 mg of α-ecdysone 22-sulfate (I) was obtained (yield on the air-dry raw material 0.001%); $C_{27}H_{43}O_6SO_3Na$, mp 224-226°C, $[\alpha]_D^{20}$ +63.3 ± 2° (c 0.91; methanol); $\lambda_{max}^{C_2H_5OH}$: 245 nm (log ε 4.15); ν_{max}^{KBr} (cm^{-1}): 3210-3345 (OH); 1652 (Δ^7-6-keto grouping), 1235 (sulfate group); CD (c 0.10, methanol): Δε = −4.19 (250 nm); Δε = +1.92 (330 nm). The nature of the cation (Na^+) was determined by the flame-photometric method. According to the results of the analysis, compound (I) contained a small amount of the potassium salt.

Mass spectrum, m/z (%): 428 (4), 410 (57), 392 (57), 380 (18), 377 (16), 359 (7), 349 (6), 336 (8), 322 (8), 312 (20), 297 (8), 295 (9), 282 (38), 267 (21), 265 (14), 255 (14), 225 (20), 211 (13), 126 (20), 109 (19), 99 (100), 81 (43), 69 (19).

α-Ecdysone (II) from (I). A solution of 5 mg of α-ecdysone 22-sulfate (I) in 5 ml of dry tetrahydrofuran was boiled on the water bath for 4 h. Then 0.5 ml of water was added to the reaction mixture and the solvent was distilled off to dryness. The residue was treated with water and the insoluble part was recrystallized from a mixture of methanol and water. This gave 2 mg of α-ecdysone with mp 234-236°C, identical with an authentic sample with respect to TLC and mass spectrum.

Sulfate ion was determined in aqueous solution with the aid of $BaCl_2$.

α-Ecdysone 2,3,25-Triacetate 22-Sulfate (III). A solution of 70 mg of α-ecdysone 22-sulfate (I) in 2 ml of pyridine was acetylated with 2 ml of acetic anhydride at room temperature for 3 days. Then the reaction mixture was diluted with water and extracted with ethyl acetate. The reaction products were chromatographed on a column of silica gel. Elution of the system with chloroform–methanol (9:1) yielded 20 mg of the triacetate (III), $C_{33}H_{49}O_9SO_3Na$, with mp 152-154°C (benzene–hexane), $[\alpha]_D^{20}$ +3.4 ± 2° (c 0.33; methanol); ν_{max}^{KBr} (cm^{-1}); 3450 (OH); 1755, 1260 (acetate group); 1675 (Δ^7-6-keto grouping); 1230 (sulfate group).

Mass spectrum, m/z (%): 512 (5), 494 (49), 479 (5), 438 (4), 414 (15), 392 (9), 385 (12), 384 (41), 383 (15), 281 (14), 255 (10), 269 (10), 249 (10), 242 (14), 225 (10), 222 (9), 173 (8), 172 (9), 109 (75), 99 (74), 81 (100), 69 (50).

SUMMARY

A new ecdysteroid, α-ecdysone 22-sulfate, has been isolated from the roots of the plant *Silene brahuica* Boiss.

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STEROL SULFATES FROM THE FAR EASTERN HOLOTHURIAN *Cucumaria japonica*

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UDC 547.922+547.925:593.96

A chloroform–methanol extract of the musculocutaneous sac of the Far-Eastern holothurian *C. japonica* has yielded a fraction of sterol sulfates (13% of the weight of the extract, 0.8% of the weight of the dry biomass), the main components of which were derivatives of cholest-5-en-3β-ol, 24-methylene-, 24-ethyl-, and 24-ethylidenecholest-5-en-3β-ols, 5α-cholestan-3β-ol, and 24-methyl- and 24-methylene-5α-cholesten-3β-ol; among the minor components were found the sulfates of 24-ethyl-5α-cholestan-3β-ol of cholesta-5,22-dien-3β-ol, of a Δ^5-C_{30} sterol, and also of dienic and trienic C_{26} sterols.

Analysis of a lipid extract of the musculocutaneous sac of the Far-Eastern holothurian *Cucumaria japonica* with the aid of GLC using specific reagents for the detection of the substances showed that the extract included two fractions the components of which contained sterol residues. One of them (fraction A) had an acidic nature and the other (B) was neutral

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Translated from Khimiya Prirodnykh Soedinenii, No. 4, pp. 470-477, July-August, 1984. Original article submitted August 24, 1983.

0009-3130/84/2004-0444$08.50