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PHYTOECDYSTEROIDS OF PLANTS OF THE GENUS *Silene*
XIV. BRAHUISTERONE FROM *Silene brahuica*

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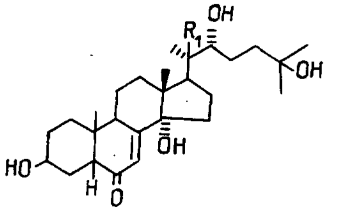
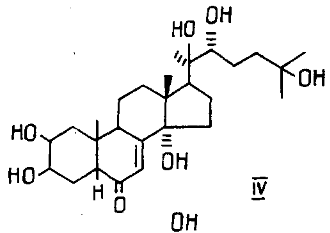
A new ecdysteroid with the composition $C_{27}H_{44}O_6$ which has been called brahuisterone has been isolated from the epigeal part of the plant Silene brahuica *Boiss. Its chemical and complete spatial structures have been established by spectral studies and the x-ray structural method (diffractometer, Cu-K_α radiations, 996 reflections, direct method, $R = 0.119$).*

Continuing a study of ecdysteroids of plants of the genus *Silene* (family Caryophyllaceae) [1, 2], from the epigeal organs of the plants *Silene brahuica* Boiss., in addition to the known 2-deoxy-α-ecdysone (I), 2-deoxyecdysterone (II), sileneoside E (III), and ecdysterone (IV), we have isolated a new ecdysteroid with the composition $C_{27}H_{44}O_6$, which we have called brahuisterone (V). The UV spectrum of this substance has an intense maximum at 243 nm (log ε 4.09) which is characteristic for 7-ene-6-ketosteroids. The IR spectrum of the ecdysteroid (V) showed a broad absorption band at 3300—3500 cm^{-1} (OH groups) and also at 1680 cm^{-1}, corresponding to a keto group conjugated with a double bond.



I. $R = R_1 = H$
II. $R = H$; $R_1 = OH$
III. $R = GLC$; $R_1 = H$



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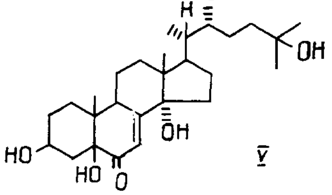

V

The mass spectrum of compound (V) contained the peak of the molecular ion with m/z 464, i.e., 16 mass units greater than that of 2-deoxy-α-ecdysone (I). The fragmentation of the side chain of the ecdysteroid (V), like that of the corresponding section of the molecule of (I) [3], was characterized by peaks of high intensity with m/z 99 and 81. This gives grounds for assuming that the side chains of both compounds have the same structure, and this was confirmed by the mutual closeness of the values of the chemical shifts of the protons of the CH_3-21, 26, and 27 groups and of H-22 in the PMR spectra of (I) and (V). Their main distinguishing feature was the absence from the spectrum of (V) of the H-5 signal and a downfield shift by 0.05 ppm of the singlet from the CH_3-19 protons (Table 1). Such a considerable shift of the protons of methyl groups is, as is known [4], observed in the PMR spectra of ecdysteroids when a 5β-OH substituent is present.

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TABLE 1. Chemical Shifts of the Protons of 2-Deoxy-α-ecdysones (I) and of Brahuisterone (V) (σ, ppm, 0 — HMDS)*

Compound	Positions of the protons and groups								
	H-3	H-5	H-7	H-9	H-22	CH_3-18	CH_3-19	CH_3-21	CH_3-26/27
I	4,00	2,87	6,13	3,42	4,00	0,61	0,95	1,17 ^3J=6,4 Гц	1,26
V	3,92	—	6,06	3,39	3,92	0,60	1,00	1,15 ^3J=6,4 Гц	1,26

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*The signals of the protons of the CH_3-21 groups of compounds (I) and (V) are doublet. In all the other cases, the signals of the protons of the methyl groups appear in the form of singlets, while, at the given working frequency of the spectrometer, H-7 forms a broadened singlet and H-3, -5, -9, and -22 give broadened multiplets.

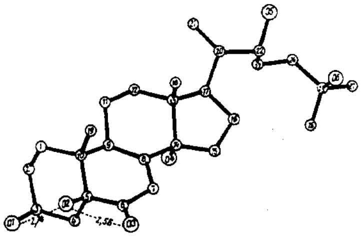

Fig. 1. Spatial structure of brahuisterone.

On the basis of these results, it was concluded that the new phytoecdysteroid under investigation, brahuisterone, was the natural 5β-OH derivative of 2-deoxy-α-ecdysone.

The x-ray structural investigation that we made unambiguously confirmed the above-proposed structure of (V) and enabled its complete spatial structure to be established. As can be seen from Fig. 1, rings A/B in the steroid part of the molecule of brahuisterone have a mutual cis- linkage and rings B/C and C/D trans- linkages.

In the structure of (V), ring A assumes a chair conformation with deviations of the C3 and C10 atoms by 0.59 and −0.71 Å, respectively. Because of the presence of the carbonyl at C6 and the double bond at C7=C8, ring B is closer to a sofa conformation with the C10 atom departing from the plane of the other five atoms. Ring C assumes the chair form with deviations of the C9 (0.51 Å) and C13 (−0.72 Å) atoms, and the five-membered ring D has the half-chair conformation (the deviation of the C13 and C14 atoms on opposite sides of the plane of the C15—C17 atom amounts to 0.37 Å).

The β-axial orientation of the hydroxy groups in the C3 and C5 positions favors the formation of an intramolecular H-bond (O1...O2 distance 2.74 Å). The distance between the oxygen atoms of the hydroxy (O2) and carbonyl (O3) groups of 2.56 Å also shows the presence of a strong intramolecular H-bond between them (Fig. 1).

The lengths of the bonds and the valence angles in the molecule of (V) are given in Table 2. The mean lengths of the C−OH (1.44 Å) and C−C and the other bonds agree well with the standard values, although the latter range within a fairly wide interval. The mean square deviations for the bond lengths and valence angles are somewhat increased (0.05 Å and 3°, respectively) because of the small number of measured reflections and the high divergence factor (see the Experimental part).

In the crystal structure of (V) an unordered molecule of the solvent — methanol — is observed. The packing of the molecules in the crystal is shown in Fig. 2, where eight minor peaks have been assigned to the atoms of the solvent molecule with a population density of 0.25. Intermolecular contacts in the crystal structure show the van der Waals distance of the atoms of the brahuisterone molecule from the unordered atoms of a methanol molecule. Intermolecular hydrogen bonds are also shown, which, binding the molecules along screw axes, form a three-dimensional carcass in the voids of which the methanol molecules are located.

Thus, on the basis of an analysis of spectral characteristics and the results of an X-ray structural investigation it has been concluded that brahuisterone is 3β,5,14α,22R,25-pentahydroxy-5β-cholest-7-en-6-one.

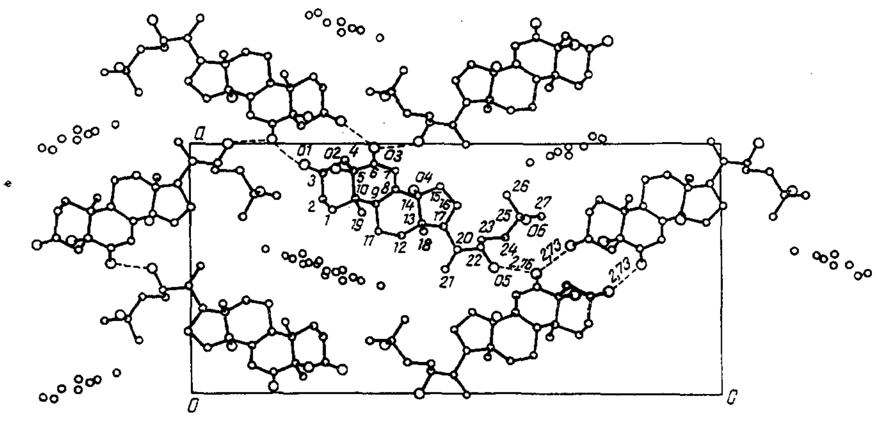

Fig. 2. Crystal structure of (V).

EXPERIMENTAL

Mass spectra were taken on a MKh 1310 instrument at an ionizing voltage of 50 V and a temperature of 100-140°C; and PMR spectra on a BS-5674 instrument (100 MHz, Tesla), C_5D_5N (δ, 0 — HMDS). For other information, see [6].

Brahuisterone. The mother liquors obtained in the isolation and recrystallization of the ecdysteroids isolated from *S. brahuica* and described previously [1] were combined and chromatographed on a column of silica gel with elution by the chloroform–ethanol (15:1) system. After crystallization from a mixture of chloroform and methanol, 10 mg of ecdysteroid was isolated with the composition $C_{27}H_{44}O_6$, mp 186-188°C, λ_{max} 2.43 nm (lg ε 4.09); ν_{max} (cm^{-1}): 3300-3500 (OH), 1680 (Δ^7-6-keto group).

Mass spectrum, m/z (%): 464 (M^+, 1), 446 (11), 431 (14), 428 (15), 418 (11), 413 (16), 410 (11), 395 (15), 378 (27), 359 (15), 348 (100), 330 (63), 300 (61), 279 (98), 99 (99), 81 (98).

X-Ray Structural Investigation. Colorless crystals of (V) in the form of elongated plates were grown from solution in methanol. The crystals were unstable — in the course of a week they were converted into a white powder. The minute amount of the substance isolated did not permit a single crystal of the desired quality to be obtained: the x-ray structural experiment was performed on a fragment of the only single crystal. Consequently, the calculations were made on a limited number of measured reflections (996). In addition, the presence in the unit cell of an unordered molecule of the solvent (methanol) likewise had an adverse influence on the quality of the experiment.

The space group, parameters of the unit cell, and intensities of the reflections from the crystal were measured on a Syntex $P2_1$ automatic four-circle diffractometer at room temperature using Cu-K_α radiation: $a = 14.509(7)$, $b = 6.205(4)$, $c = 31.41(2)$ Å, $d_{calc} = 1.162$ g/cm^3; space group $P2_12_12_1$, Z = 4.

The intensities of reflection with $\theta < 58°$ were obtained on the above-mentioned diffractometer ($\theta/2\theta$ scanning). In the primary treatment weak reflections with $I < 2\sigma(I)$ were excluded. In the calculations we used 747 reflections with $|F| > 4\sigma(|F|)$. The structure was determined by the direct method using the SHELXS-86 program [7] with the TEXP procedure, which permitted a partial structure to be included in the refining tangent of the direct method. The structure was refined by the method of least squares (MLS) in the full-matrix isotropic approximation by the SHELX-76 program [8] (both programs in the PC MSDOS version). The positions of the H atoms were calculated in the final stage of refinement. The final value of the discrepancy factor R = 0.119. The coordinates of the nonhydrogen atoms of the structure of (V) are given in Table 3.

TABLE 2. Bond Lengths r (Å) and Valence Angles ω (degrees) in the Structure of (V)

Bond	r	Bond	r	Angle	ω	Angle	ω	Angle	ω
C2—C1	1,55	C13—C12	1,51	C10—C1—C2	113	C11—C9—C8	119	O4—C14—C13	100
C10—C1	1,59	C14—C13	1,65	C3—C2—C1	108	C11—C9—C10	110	O4—C14—C15	113
C3—C2	1,52	C17—C13	1,52	C4—C3—C2	118	C5—C10—C1	109	C16—C15—C14	104
C4—C3	1,56	C18—C13	1,58	O1—C3—C2	112	C9—C10—C1	110	C17—C16—C15	110
O1—C3	1,52	C15—C14	1,50	O1—C3—C4	106	C9—C10—C5	107	C20—C17—C13	122
C5—C4	1,52	O4—C14	1,46	C5—C4—C3	110	C19—C10—C1	106	C20—C17—C16	115
C6—C5	1,46	C16—C15	1,53	C6—C5—C4	109	C19—C10—C5	113	C21—C20—C17	112
C10—C5	1,60	C17—C16	1,62	C10—C5—C4	111	C19—C10—C9	109	C22—C20—C17	112
O2—C5	1,43	C20—C17	1,54	C10—C5—C6	110	C12—C11—C9	111	C22—C20—C21	113
C7—C6	1,47	C21—C20	1,50	O2—C5—C4	110	C13—C12—C11	111	C23—C22—C20	112
O3—C6	1,19	C22—C20	1,53	O2—C5—C6	107	C14—C13—C12	110	O5—C22—C20	108
C8—C7	1,29	C23—C22	1,53	O2—C5—C10	106	C17—C13—C12	117	O5—C22—C23	107
C9—C8	1,57	O5—C22	1,39	C7—C6—C5	120	C17—C13—C14	103	C24—C23—C22	115
C14—C8	1,54	C24—C23	1,54	O3—C6—C5	120	C18—C13—C12	110	C25—C24—C23	115
C10—C9	1,65	C25—C24	1,61	O3—C6—C7	119	C18—C13—C14	105	C26—C25—C24	110
C11—C9	1,61	C26—C25	1,50	C8—C7—C6	120	C18—C13—C17	109	C27—C25—C24	106
C19—C10	1,54	C27—C25	1,60	C9—C8—C7	127	C13—C14—C8	112	C27—C25—C26	102
C12—C11	1,56	O6—C25	1,43	C14—C8—C7	120	C15—C14—C8	118	O6—C25—C24	108
				C14—C8—C9	109	C15—C14—C13	101	O6—C25—C26	114
				C10—C9—C8	105	O4—C14—C8	108	O6—C25—C27	112

TABLE 3. Coordinates ($\times 10^4$) of the Nonhydrogen Atoms in the Structure of (V)

Atom	x	y	z	Atom	x	y	z
C1	7316(20)	1770(52)	2680(8)	C18	6433(20)	2567(51)	4386(9)
C2	7767(15)	—0332(45)	2512(8)	C19	7226(21)	4625(58)	3243(9)
C3	8805(19)	0001(64)	2500(10)	C20	5738(15)	—0867(43)	5041(7)
C4	9291(23)	0827(61)	2913(10)	C21	4944(20)	—1815(55)	4799(9)
C5	8823(17)	2871(49)	3071(7)	C22	5849(26)	—1803(62)	5490(10)
C6	9234(18)	3535(48)	3476(8)	C23	6090(21)	—4210(51)	5485(8)
C7	8902(23)	2604(61)	3877(10)	C24	6168(19)	—5276(57)	5925(8)
C8	8157(21)	1476(52)	3883(9)	C25	7044(23)	—4549(62)	6206(10)
C9	7567(23)	0706(68)	3493(11)	C26	7930(22)	—5006(68)	5974(10)
C10	7736(21)	2545(58)	3121(9)	C27	7096(24)	—6162(58)	6602(9)
C11	6483(18)	0278(60)	3556(8)	O1	9098(13)	1521(34)	2147(6)
C12	6283(19)	—0945(51)	3979(7)	O2	8952(12)	4579(32)	2773(5)
C13	6747(21)	0139(63)	4353(9)	O3	9800(14)	4926(41)	3483(6)
C14	7877(19)	0210(56)	4283(9)	O4	8073(13)	—2074(35)	4214(6)
C15	8204(19)	1024(52)	4708(8)	O5	5018(14)	—1584(37)	5708(6)
C16	7539(22)	0005(68)	5029(11)	O6	6923(18)	—2361(48)	6333(7)
C17	6646(16)	—0942(47)	4785(7)				

Fixed positions of the atoms of an unordered methanol molecule (occupancy of the positions 0.25)

Atom	x	y	z	Atom	x	y	z
A1	10166(78)	—07354(212)	7760(32)	A5	10242(51)	—10140(159)	7564(25)
A2	09473(55)	—06401(150)	7125(25)	A6	09851(57)	—08707(146)	7412(24)
A3	09628(57)	—01752(167)	6926(27)	A7	09261(74)	—10121(214)	6410(34)
A4	09506(58)	—10283(198)	6788(28)	A8	09860(55)	—10065(153)	7128(24)

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