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5β(H)-AUSTRICIN, A NEW GUAIANOLIDE FROM *Artemisia leucodes*

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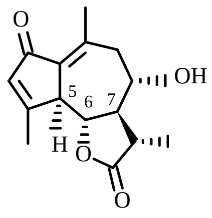
*A new sesquiterpene lactone 5β(H)-austricin was isolated from white wormwood (*Artemisia leucodes *Schrenk.). Its structure was established by chemical and spectral methods.*

Key words: sesquiterpene lactones, 5β(H)-austricin, relactonization reaction.

The sesquiterpene lactones austricin (**1**) leucomisin, parishin C, anhydroaustricin, matricarin, and artelin were isolated previously from flowerheads and leaves of *Artemisia leucodes* Schrenk. (white wormwood) [1-5]. In continuation of research of lactones of the ethanol extract of the aerial part of this plant, we isolated from polar fractions a new sesquiterpene lactone (**2**), $C_{15}H_{18}O_4$, M^+ 262, mp 258-260°C (EtOAc), $[\alpha]_D^{25}$ +147.06 (*c* 0.034, $CHCl_3$).

The IR spectrum of **2** has absorption bands at 3287 (OH), 1793 (γ-lactone carbonyl), 1664 (α,β-unsaturated ketone), and 1627 and 1610 cm^{-1} (double bonds).

The PMR spectra of **2** (Table 1) and austricin [1] are practically identical with the exception of the signal for H-6, which appears as a doublet of doublets at 4.2 ppm with SSCC $J_{5,6}$ = 4.0 and $J_{6,7}$ = 7.0 Hz. Therefore, it can be assumed that the pairs H-5—H-6 and H-6—H-7 are in *cis*-positions relative to each other. Therefore, **2** can be formulated as 8α-hydroxy-2-oxo-$5\alpha,6\alpha,7\alpha,11\beta$(H)-guai-1(10),3(4)-dien-6,12-olide, which has structure **2a**.



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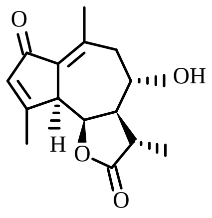
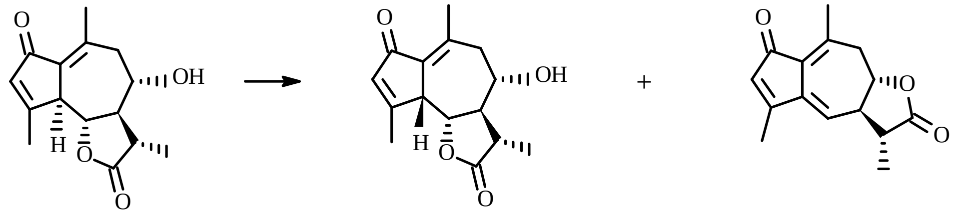


2a

It should be noted that a similar difference in the spectra was described earlier for badkhysin and isobadkhysin [6, 7], where relactonization by base led to isomerization of the C-5 proton with retention of configuration of the lactone ring.

The structure of **2** was refined by saponfying austricin. Hydrolysis of **1** by K_2CO_3 solution (0.5 N) in CH_3OH with subsequent acidification by HCl (0.5%) gave two products (**2b** and **3**).



1 **2b** **3**

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TABLE 1. PMR Spectra of **2** and **3** ($CDCl_3$, 0 = TMS, δ, ppm)

Atom	^{1}H chemical shift (SSCC Hz)	
	2	**3**
H-3	6.12 m	6.10 q (J = 2.0)
H-5	3.45 br.d (J_1 = 4.0)	-
H-6	4.20 dd (J_1 = 7.0; J_2 = 4.0)	5.81 d (J = 3.0)
H-7	1.97 ddd (J_1 = 12.0; J_2 = 10.5; J_3 = 7.0)	2.74 ddd (J_1 = 13.0; J_2 = 10.0; J_3 = 4.0)
H-8	4.58 ddd (J_1 = 10.5; J_2 = 10.0; J_3 = 6.0)	4.34 ddd (J_1 = 11.0; J_2 = 10.0; J_3 = 4.0)
H-9a	2.47 dd (J_1 = 19.0; J_2 = 10.0)	3.07 dd (J_1 = 19.0; J_2 = 4.0)
H-9b	3.27 dd (J_1 = 19.0; J_2 = 6.0)	2.86 dd (J_1 = 19.0; J_2 = 11.0)
H-11	2.60 dq (J_1 = 12.0; J_2 = 7.0)	2.58 dq (J_1 = 13.0; J_2 = 7.0)
H-13	1.40 d (J_1 = 7.0)	1.38 d (J = 7.0)
H-14	2.27 s	2.43 s
H-15	2.13 s	2.13 s

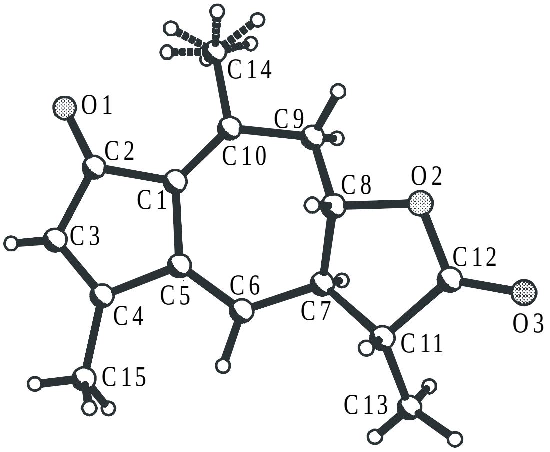

Fig. 1. Crystal structure **3**.

The main product was a colorless crystalline compound, $C_{15}H_{18}O_4$, mp 258-260°C (EtOAc). Comparison of spectral data and the mixed melting point with an authentic sample showed that **2b** was identical to **2**, which we isolated previously from white wormwood.

Therefore, sesquiterpene **2** is a new compound with structure **2b**, 5β(H)-austricin (8α-hydroxy-2-oxo-7α,5β,6β,11β(H)-guai-1(10),3(4)-dien-6,12-olide).

The second product was a colorless crystalline compound (**3**), $C_{15}H_{16}O_3$, mp 193-195°C (EtOAc), M^+ 244.

The IR spectrum of **3** contains absorption bands at 1775 (γ-lactone carbonyl), 1689 (conjugated ketone of a five-membered ring), and 1623 and 1600 cm^{-1} (double bonds). It should be noted that absorption bands typical of OH are absent.

The UV spectrum has an absorption maximum characteristic of a conjugated system: λ_{max} 244, 257, and 308 nm with log ε 5.42, 5.41, and 4.79, respectively.

Table 1 lists the PMR spectrum, which lacks a signal characteristic of the C-5 proton. However, the signal of H-6 is shifted by 1.61 ppm to weak field. Obviously, this is due to the formation of an olefinic bond at C-6—C-5 as a result of opening of the lactone ring by base and closure at C-8 after acidification.

Comparison of the IR, UV, mass, and PMR spectra of **3** with literature data [8] identified it as dehydroisoleucomisin, which was prepared previously by hydrolysis of austricin.

TABLE 2. Bond Lengths (d, Å)

Bond	d	Bond	d
O1C2	1.226 (6)	C4C15	1.478 (8)
C1C10	1.344 (8)	C5C6	1.350 (7)
C1C5	1.494 (7)	C6C7	1.470 (7)
C1C2	1.498 (8)	C7C8	1.506 (8)
O2C12	1.349 (7)	C7C11	1.527 (8)
O2C8	1.464 (7)	C8C9	1.486 (9)
C2C3	1.459 (9)	C9C10	1.507 (9)
O3C12	1.216 (7)	C10C14	1.516 (8)
C3C4	1.330 (8)	C11C12	1.496 (8)
C4C5	1.460 (6)	C11C13	1.509 (10)

TABLE 3. Bond Angles (ω, deg)

Angle	ω	Angle	ω
C10C1C5	131.0 (5)	C6C7C11	116.0 (5)
C10C1C2	124.4 (5)	C8C7C11	101.8 (4)
C5C1C2	104.5 (4)	O2C8C9	108.6 (5)
C12O2C8	109.2 (4)	O2C8C7	103.6 (5)
O1C2C3	125.1 (6)	C9C8C7	117.0 (6)
O1C2C1	128.2 (6)	C8C9C10	115.8 (5)
C3C2C1	106.6 (4)	C1C10C9	126.2 (5)
C4C3C2	111.2 (5)	C1C10C14	122.2 (6)
C3C4C5	110.4 (5)	C9C10C14	11.6 (5)
C3C4C15	127.0 (5)	C12C11C13	112.5 (6)
C5C4C15	122.5 (5)	C12C11C7	100.8 (5)
C6C5C4	123.3 (5)	C13C11C7	117.4 (6)
C6C5C1	129.4 (4)	O3C12O2	120.2 (5)
C4C5C1	107.2 (4)	O3C12C11	129.2 (6)
C5C6C7	125.7 (5)	O2C12C11	110.5 (5)
C6C7C8	113.7 (5)		

In view of the fact that the orientation of the C-11 methyl was not previously defined, we carried out an x-ray structural analysis of **3**. Figure 1 shows the molecular structure. The bond lengths are slightly shortened (Table 2) and the bond angles (Table 3) are significantly distorted compared with the usual values [9]. The difference is from 0.9 (O2–C8–C9) to 15.5° (C5–C1–C2).

The lactone ring is *trans*-fused to the guaiane skeleton. The torsion angle H7–C7–C8–H8 is 180.0°. The conformation of the lactone ring is almost an ideal envelope ($\Delta C_S^7 = 1.1°$). Atom C-7 deviates from the plane of the remaining atoms (coplanar within ±0.006 Å) by 0.58 Å. The seven-membered ring (C5,C6,C7,C8,C9,C10,C1) adopts a distorted 8-sofa conformation ($\Delta C_S^8 = 12.9°$) with deviations of C8, C9, and C14 from the plane of C5, C6, C7, C10, and C1, which are coplanar within 0.007 Å, by -0.84, -0.22, and 0.27 Å, respectively. The five-membered ring (C1,C2,C3,C4,C5) is planar with O1 and C15 also in this plane.

Our investigation and previous ones [10] confirmed that the orientation of the methyl group does not change as a result of relactonization.

TABLE 4. Atomic Coordinates (× 10^4; for H, × 10^3)

Atom	x	y	z	Atom	x	y	z
O1	2596 (9)	-296 (2)	2604 (2)	C14	3646 (13)	-355 (4)	1316 (4)
C1	2845 (8)	928 (3)	1924 (2)	C15	2102 (10)	2647 (4)	3112 (3)
O2	2437 (8)	2127 (3)	79 (2)	H3	212 (6)	96 (3)	336 (2)
C2	2581 (10)	461 (3)	2520 (3)	H6	270 (8)	301 (3)	203 (3)
O3	2655 (7)	3343 (3)	-416 (2)	H7	457 (6)	252 (2)	101 (2)
C3	2287 (11)	1087 (4)	3003 (3)	H8	110 (6)	183 (2)	79 (2)
C4	2306 (8)	1854 (3)	3768 (2)	H9A	275 (13)	68 (5)	40 (4)
C5	2672 (7)	1826 (3)	2101 (2)	H9B	448 (11)	101 (4)	64 (3)
C6	2898 (7)	2517 (3)	1749 (2)	H11	135 (7)	336 (3)	78 (2)
C7	3299 (8)	2527 (3)	1080 (2)	H13A	317 (11)	453 (5)	49 (3)
C8	2392 (9)	1834 (4)	723 (2)	H13B	388 (9)	426 (4)	113 (3)
C9	3261 (12)	997 (4)	751 (3)	H13C	513 (14)	394 (5)	65 (4)
C10	3196 (8)	566 (3)	1373 (3)	H15A	103 (8)	299 (3)	299 (2)
C11	2662 (10)	3298 (4)	724 (3)	H15B	194 (6)	252 (3)	351 (2)
C12	2617 (9)	2966 (4)	74 (3)	H15C	305 (12)	302 (5)	312 (4)
C13	3797 (14)	4080 (5)	780 (3)				

EXPERIMENTAL

Melting points were determined on a Boetius apparatus. IR spectra were recorded (in KBr disks) on an Avatar 360 instrument (Thermo Nicolet); UV spectra (EtOH solutions), on a Specord UV VIZ instrument. NMR spectra were recorded on a DRX-500 spectrometer (Bruker) at working frequencies 500.13 MHz (^{1}H) and 125.76 MHz (^{13}C) in $CDCl_3$. High resolution mass spectra (EI, 70 eV) were obtained in an MAT 8200 (Finnigan). Optical rotations were measured on a CM-2 polarimeter.

We used SiO_2 (KSK) for column chromatography and Silufol plates for TLC with development by $KMnO_4$ solution (1%).

The aerial part of white wormwood was collected in 2000 year during budding (August) in the Moiyn-Kum desert (Kazakhstan), dried in air, and ground.

Starting austricin (**1**) with mp 147-149°C, $[\alpha]_D^{20}$ +28° (*c* 0.1, $CHCl_3$) was isolated as before [2].

Isolation of 5*β*(H)-Austricin (2). Raw material (*A. leucodes* Schrenk., 1 kg) was extracted twice with EtOH (2 × 8 L) at 60°C. The combined EtOH extracts were filtered and evaporated in vacuum in a rotary evaporator. The resulting dry extract (210 g) was treated with EtOH:H_2O (0.9 L, 2:1 by vol.), thoroughly mixed, and filtered to remove solids. The filtrate was extracted with $CHCl_3$ (3 × 0.2 L). The $CHCl_3$ extracts were combined and evaporated to dryness. The resulting solid (93 g) was chromatographed over SiO_2 with elution by benzene and then benzene:ethylacetate (7:3). Polar fractions (74-101) were combined and evaporated to dryness. The resulting solid (5 g) was repeatedly chromatographed over SiO_2 (50 g) with elution by benzene:ethylacetate (60:40) to isolate **2** (40 mg, 0.004%), $C_{15}H_{18}O_4$, M^+ 262, mp 258-260°C (EtOAc), $[\alpha]_D^{25}$ +147.06 (*c* 0.034, $CHCl_3$).

UV spectrum (EtOH, λ_{max}, nm): 255 (log ε 5.29). IR spectrum (KBr, ν, cm^{-1}): 3287 (OH), 1793 (γ-lactone C=O), 1664 (C=O), and 1627 and 1610 (C=C).

^{13}C NMR spectrum (125.76 MHz, $CDCl_3$): 196.46 (C-2), 177.65 (C-12), 168.16 (C-4), 145.16 (C-10), 135.61 (C-3), 128.41 (C-1), 73.96 (C-6), 51.64 (C-5), 45.44 (C-9), 42.75 (C-11), 19.70 (C-15), 16.64 (C-14), 14.32 (C-13).

Table 1 gives the PMR spectrum.

Saponification of 1. A solution of **1** (200 mg, 0.76 mmol) in MeOH (10 mL) at room temperature was treated with K_2CO_3 (1 mL, 1 M, 1 mmol) and stirred for 3 h. Then solvent was removed in vacuum. The solid was dissolved in EtOAc, washed with HCl (0.5%) and water until the washings were neutral, and dried over Na_2SO_4. The solvent was removed in vacuum. The solid was chromatographed (gradient elution by petroleum ether:ethylacetate, 100:0 → 50:50) to afford lactone **3** (0.07 g, 35%) and **2** (0.105 g, 52.2%).

Dehydroisoleucomisin (3). mp 193-195°C (EtOAc), lit. mp 203-205°C.

UV spectrum (EtOH, λ_{max}, nm): 244, 257, 308 (log ε 5.42, 5.41, 4.79, respectively. IR spectrum (KBr, ν, cm^{-1}): 1775 (γ-lactone C=O), 1689 (C=O), and 1623 and 1600 (C=C).

Table 1 gives the PMR spectrum.

X-ray Structure Analysis. Cell constants and intensities of 1283 independent reflections were measured on a Bruker P4 diffractometer (Mo Kα, graphite monochromator, θ/2θ-scanning, 2θ < 50°). The crystals are orthorhombic: $a = 7.3555(6)$, $b = 16.0139(18)$, $c = 21.5122(19)$ Å, $\alpha = \beta = \gamma = 90.0°$, V = 2533.9(4) $Å^3$, $d_{calc} = 1.281$ g/cm^3, Z = 8 ($C_{15}H_{16}O_3$), space group $C222_1$. The structure was solved by direct methods and refined by anisotropic full-matrix least squares methods for nonhydrogen atoms. All H atoms except for H14a, H14b, and H14c were found in a difference synthesis and refined isotropically. The H atoms of $C^{14}H_3$ were fixed in two positions of different occupancy (0.4 and 0.6) owing to disorder. We used 1002 reflections with $I > 2\sigma(I)$ in the calculations. The final agreement factors were R = 0.0564 and $R_w = 0.1713$. The structure was solved and refined using SHELXS-97 and SHELXL-97 programs. Table 4 contains the atomic coordinates.

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