Chemistry of Natural Compounds, Vol. 36, No. 3, 2000

9β-HYDROXY-11β(H),13-DIHYHDROSANTAMARINE, A NEW EUDESMANOLIDE FROM *Artemisia leucotricha*

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UDC 547.314+548.737

A new sesquiterpene lactone, 9β-hydroxy-11β(H),13-dihydrosantamarine, and known terpenes are isolated from the underground part of Artemisia leucotricha *Krasch ex Ladyg (Asteraceae). The structure is determined by spectral and x-ray structural analysis.*

Key words: *Artemisia leucotricha* (Asteraceae), sesquiterpene lactone, 9β-hydroxy-11β(H),13-dihydrosantamarine, x-ray structural analysis.

According to the literature [1], the *Artemisia* L. genus is one of the largest in the Asteraceae family (compound flower). The flora of eastern Pamir has 17 species that are insufficiently studied chemically [2]. Therefore, we investigated the underground parts of *Artemisia leucotricha* Krasch ex Ladyg, one of the most common species of *Artemisia* in eastern Pamir [1, 3].

More than 20 secondary plant metabolites are found by TLC of the $CHCl_3$ extract of the underground part (after removal of extraneous substances). Column chromatography (silica gel) of the total extracted substances isolated 11 crystalline products, denoted in order of increasing polarity as compounds **1-11**.

Compounds **1** and **3-5** are a monoterpene, triterpene, and a sterol. They were identified as camphor (**1**), β-amyrin acetate (**3**), β-amyrin (**4**), and β-sitosterol (**5**), respectively [4, 5].

Compounds **6**, **8**, **9**, and **11** were identified as sesquiterpene lactones based on the presence in their IR spectra of a characteristic absorption band for the γ-lactone carbonyl group at 1760-1790 cm^{-1} [6].

The present article presents data establishing the structure of lactone **11**.

Compound **11** has the formula $C_{15}H_{22}O_4$, mp 201-202°C, M^+ 266. The IR spectrum of lactone **11** exhibits a broad band with a split maximum at 3288 and 3277 cm^{-1} that corresponds to absorption by a H-bonded hydroxyl group, a strong carbonyl absorption band for the γ-lactone ring at 1784 cm^{-1}, and a weak band at 1650 cm^{-1} that is characteristic of an isolated double bond.

The mass spectrum of **11** contains a peak for the molecular ion with *m/z* 266 $[M]^+$ and peaks for ions with *m/z* 248 $[M - H_2O]^+$, 233 $[248 - CH_3]^+$, and 230 $[248 - H_2O]^+$, that are due to loss of two water molecules and one methyl group. In addition, fragments (see Experimental) characteristic of decomposition by electron impact of the eudesmane sesquiterpene lactones are observed [7].

The PMR spectrum in C_5D_5N contains signals of two tertiary methyl groups (1.11 ppm, 3H, s; 1.78 ppm, 3H, br. s), a secondary methyl group (1.05 ppm, 3H, d, J = 7 Hz), an olefin (5.20 ppm, 1H, br. s), and two hydroxyl protons (6.18 ppm, 1H, br. s and 7.1 ppm, 1H, br. s). Signals of the lactone and two geminal hydroxyl protons are superimposed and evident as a 3-proton multiplet centered at 3.95 ppm. These signals for the hydroxyl protons disappear upon adding CF_3CO_2H whereas the 3-proton signal at 3.95 ppm changes significantly, confirming the superimposition of the signals of the geminal hydroxyl and lactone protons.

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0009-3130/00/3603-0284$25.00

TABLE 1. Bond Lengths (r, Å) and Angles (ω, deg) in molecule **11**

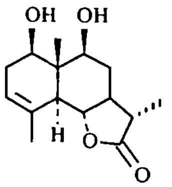
Bond	r	Angle	ω	Angle	ω
O1-C1	1.433(3)	C12-O3-C6	107.6(2)	C8-C7-C11	121.9(2)
O2-C9	1.431(3)	O1-C1-C2	111.9(2)	C7-C8-C9	107.3(2)
O3-C12	1.348(4)	O1-C1-C10	111.7(2)	O2-C9-C8	107.4(2)
O3-C6	1.455(3)	C2-C1-C10	111.1(2)	O2-C9-C10	112.7(2)
O4-C12	1.199(4)	C3-C2-C1	112.4(2)	C8-C9-C10	113.7(2)
C1-C2	1.514(4)	C4-C3-C2	124.5(2)	C14-C10-C1	110.5(2)
C1-C10	1.547(4)	C3-C4-C15	120.2(2)	C14-C10-C9	109.7(2)
C2-C3	1.499(4)	C3-C4-C5	120.6(2)	C1-C10-C9	110.0(2)
C3-C4	1.329(4)	C15-C4-C5	119.1(2)	C14-C10-C5	112.7(2)
C4-C15	1.495(4)	C6-C5-C4	118.1(2)	C1-C10-C5	104.4(2)
C4-C5	1.521(3)	C6-C5-C10	106.5(2)	C9-C10-C5	109.4(2)
C5-C6	1.523(4)	C4-C5-C10	111.5(2)	C12-C11-C13	113.4(3)
C5-C10	1.568(3)	O3-C6-C7	102.5(2)	C12-C11-C7	99.4(2)
C6-C7	1.511(4)	O3-C6-C5	114.9(2)	C13-C11-C7	117.3(2)
C7-C8	1.516(4)	C7-C6-C5	111.4(2)	O4-C12-O3	121.1(3)
C7-C11	1.523(4)	C6-C7-C8	111.1(2)	O4-C12-C11	127.5(3)
C8-C9	1.522(4)	C6-C7-C11	101.0(2)	O3-C12-C11	111.4(2)
C9-C10	1.546(3)				
C10-C14	1.535(3)				
C11-C12	1.508(4)				
C11-C13	1.520(4)				

TABLE 2. Atomic Coordinates ($\times 10^4$) and Equivalent Thermal Parameters U_{eq}(Å$^2 \times 10^3$) in molecule **11**

Atom	x	y	z	U_{eq}
O1	1334(4)	2942(3)	7492(1)	64(1)
O2	1978(4)	6038(3)	7526(1)	56(1)
O3	6175(3)	5338(2)	9516(1)	49(1)
O4	8217(4)	7062(3)	9898(1)	84(1)
C1	2446(4)	2707(3)	7994(1)	43(1)
C2	1633(5)	1383(4)	8354(1)	51(1)
C3	2554(4)	1317(3)	8925(1)	46(1)
C4	3719(4)	2427(3)	9132(1)	37(1)
C5	4244(4)	3881(3)	8785(1)	32(1)
C6	4651(4)	5430(3)	9095(1)	35(1)
C7	5404(4)	6693(3)	8699(1)	36(1)
C8	3894(4)	7131(3)	8263(1)	39(1)
C9	3364(4)	5618(3)	7948(1)	38(1)
C10	2651(4)	4262(3)	8335(1)	34(1)
C11	6196(4)	7904(3)	9116(1)	41(1)
C12	6993(5)	6788(4)	9555(1)	50(1)
C13	7681(5)	9084(4)	8897(1)	57(1)
C14	706(4)	4723(4)	8601(1)	47(1)
C15	4621(4)	2213(4)	9699(1)	51(1)

The spectral characteristics (IR and mass spectrometry and PMR) and the different aggregate state suggest that the isolated eudesmanolide **11** is a stereoisomer of 9α-hydroxy-11β(H),13-dihydrosantamarine [8].

The structure was unambiguously determined by an x-ray structural analysis, according to which rings *A*/*B* and *B*/*C* are *trans*-fused in the tricyclic system of eudesmanolide **11**. The half-chair conformation is characteristic for eudesmanolides containing a double bond in the C3=C4 position of ring *A* [7]. Therefore, ring *A* adopts the rarely encountered 10β-half-chair conformation that is favored not only by the presence of the C3=C4 double bond in the ring but also by the mutual placement



11

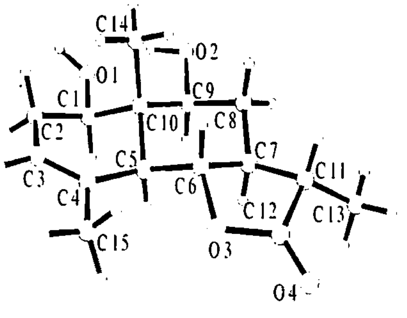


Fig. 1. Structure of molecule **11**.

of the neighboring hydroxyl and methyl groups. The cyclohexane ring *B* has the usual chair conformation. The lactone ring has the 7α-envelope conformation at the 6,7-*trans*-position.

A general view of molecule **11** is shown in Fig. 1.

The C1 and C9 hydroxyls are β-equatorial. The C10 methyl group is β-axial. The parallel (*syn*) orientation of the hydroxyls favors the formation of intramolecular H-bonds O1...H-O2. This is consistent with the distances O1...O2, 2.69 Å and O1-H...O2, 1.76 Å and the angle O1–H–O2, 150.1 . The methyl group in the lactone ring (C13) has the α-equatorial orientation.

The Csp^3–Csp^3 bond lengths (varying from 1.511-1.547 Å) are similar to the generally accepted value of 1.540 Å, except for the C5–C10 distance [1.568(3) Å] (Table 1). The increased C5–C10 bond length is characteristic of eudesmane sesquiterpenoids [7, 9-10]. The C3=C4 and C12=O4 double bonds have lengths of 1.329(4) and 1.199(4) Å, respectively. However, the lengths of the heterobonds O1–C1 [1.433 (3) Å] and O2–C9 [1.431(3) Å] are practically identical. These values within experimental uncertainty correspond to the standard values [11]. The bond lengths of other bonds are normal. The bond angles are typical of those observed in other tricyclic eudesmanolides [7, 9-10].

The packing and intermolecular contacts are consistent with O...H–O H-bonds. The O2...O1 distance (2.79 Å) between atoms of two molecules are related by a screw translation along the b axis. This means that the active H of O1 interacts with the unshared pair of O2 because the H of O2 is confined within the molecule by the unshared pair of O1.

Thus, it is unambiguously established that the isolated eudesmanolide **11** is a stereoisomer of 9α-hydroxy-11β(H),13-dihydrosantamarine at the asymmetric C9 and has the structure 1,9β-dihydroxy-3,4-en-5,7α,6,11β(H)-eudesm-6,12-olide.

EXPERIMENTAL

IR spectra were recorded on a Perkin—Elmer 2000 IR-Fourier spectrometer (KBr pellets). Mass spectra were measured on a Kratos GB MS-25 RF chromatography-mass spectrometer. ^1H NMR spectra were recorded on a Tesla BS 567 A specrometer at 100 MHz. The internal standard was HMDS. Chemical shifts are reported in the σ-scale.

Isolation Method. The subterrean part of *A. leucoltricha* (6 kg) that was collected in 1994 during flowering on August 2-4 near Kyzylrabat of Murgabskii region in the Republic of Tadzhikistan was extracted five times with $CHCl_3$ (1:5, material:solvent). The combined extracts were evaporated and treated with alcohol (90%). The precipitate was filtered after one day. The filtrate was evaporated. The solid (170 g) was placed on a silica-gel column in a 1:20 ratio. Elution used benzine and its mixtures with ethylacetate, ethylacetate, and a ethylacetate—methanol mixture. Fractions (550 ml) from chromatographic separation were collected in the following order: 1-11 (benzine), 12-20 (benzine—ethylacetate, 49:1), 21-73 (benzine—ethylacetate, 9:1), 74-93 (benzine—ethylacetate, 4:1), 94-181 (benzine—ethylacetate, 2:1), 182-185 (ethylacetate), 186-236 (ethylacetate—methanol, 1:1).

Chromatographic separation isolated 11 pure substances: from fractions 4-9 (**1**), 13-15 (**2**), 21-23 (**3**), 27-30 (**4**), 34-35 (**5**), 39-40 (**6**), 41-42 (**7**), 44-50 (**8**), 80-88 (**9**), 94-102 (**10**), 183-185 (**11**).

Camphor (1). $C_{10}H_{16}O$, mp 177-178 C, M^+ 152. The monoterpene was identified by mass spectra and direct comparison with an authentic sample.

β-Amyrin Acetate (3). $C_{32}H_{52}O_2$, mp 239-240 C, M^+ 468. The triterpene was identified by mass spectra and by direct comparison with an authentic sample.

β-Amyrin (4). $C_{30}H_{50}O$, mp 197-198° C, M^+ 426. The triterpene was identified by mass spectra and by direct comparison with an authentic sample.

β-Sitosterol (5). $C_{29}H_{50}O$, mp 137-139° C, M^+ 414. The sterol was identified by mass spectra and by direct comparison with an authentic sample.

9β-Hydroxy-11β(H),13-dihydroxysantamarine (11). $C_{15}H_{22}O_4$, mp 201-202° C. Mass spectrum, m/z (I_{rel}, %): M^+ 266 (22), 248 (M^+ - H_2O, 58), 233 (248 - CH_3, 8), 230 (248 - H_2O, 10), 220 (9), 215 (6), 204 (13), 197 (10), 193 (18), 176 (10), 175 (44), 173 (6), 166 (11), 165 (37), 164 (27), 150 (10), 136 (40), 108 (21), 107 (70), 106 (23), 97 (41), 84 (82), 55 (100).

X-ray Study. The cell parameters and intensities of 1872 independent reflections were measured on a Nonius CAD-4 diffractometer (Mo Kα-radiation, graphite monochromator, Θ/2Θ-scanning). Crystals are orthorhombic, a = 6.913(3), b = 8.419(2), c = 23.759 (12) Å, V = 1382.7(10) Å^3, d_{calc} = 1.279 g/cm^3, Z = 4 ($C_{15}H_{22}O_4$), space group $P2_12_12_1$. The structure was solved by direct methods and refined by anisotropic full-matrix least-squares methods for nonhydrogen atoms. All H atoms, with the exception of the hydroxyl H, which was found in a difference electron-density synthesis, were assigned geometrically and fixed on "rider" type. A total of 1424 reflections with $I > 2\sigma(I)$ was used in the calculation. The final discrepancy factors are R = 0.0422 and R_w = 0.1343. Atomic coordinates are given in Table 2. All calculations were performed on an IBM-486 PC using the SHELXTL and SHELXL-95 programs.

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