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STRUCTURE OF ISKENDEROLIDE

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The structure of a sesquiterpene lactone germacranolide was defined as 1β,6β-dihydroxygermacr-4(5),10(14)-dien-8,12-olide and was the C_6-epimer of (11R)-11,13-dihydrotatridin-B.

Keywords: *Artemisia iskenderiana* Rzazade, Asteraceae, sesquiterpene lactone, germacranolide, PMR, ^{13}C NMR, DEPT 135, DEPT 90 spectra.

We continued research on the structure of a new sesquiterpene lactone isolated from the aerial part of *Artemisia iskenderiana* Rzazade, a new sage species described by Rzazade [1] but not recognized by Polyakov [2, 3].

The composition of iskenderolide was $C_{15}H_{22}O_4$, mp 190–191°C. The IR spectrum showed absorption bands for hydroxyls (3300 cm^{-1}), a γ-lactone carbonyl (1755), and double bonds (1665, 1650). The appearance in the IR spectrum of a strong band at 910 cm^{-1} indicated that the molecule might contain a methylene double bond [4].

The PMR spectrum of the compound exhibited a doublet for a secondary methyl on a lactone ring (1.42 ppm, J = 7 Hz, CH_3–CH<) and a singlet characteristic of a vinylmethyl (1.60 ppm). Weakly split (by ~1.5 Hz) 1H doublets at 5.13 and 5.40 ppm belonged to a H_2C=C< group.

The appearance in the spectrum of resonances for exomethylene and vinylmethyl groups in addition to a doublet for an olefinic proton (5.28 ppm, J = 9.65 Hz) suggested that the molecule contained methylene and secondary–tertiary double bonds. A 2H resonance at 3.84 ppm was apparently the result of the overlap of lactone and gem-hydroxyl protons. The gem-hydroxyl proton of a second OH group appeared in the spectrum as a triplet at 4.35 ppm (1H, J = 9.65 Hz).

According to ^{13}C NMR, ^{13}C DEPT 135, ^{13}C DEPT 90, and ^{13}C DEPT 45 spectra, iskenderolide was based on a germacrane C skeleton and not guaiane, as proposed earlier [5]. The ^{13}C NMR spectrum taken with full suppression of proton spin–spin coupling showed 15 singlets (15.0, 17.0, 35.0, 36.0, 42.0, 43.0, 58.0, 72.0, 76.0, 83.0, 112.0, 129.0, 137.0, 152.0, and 180.0 ppm) that were consistent with the presence in the molecule of 15 C atoms. According to ^{13}C DEPT 45 and ^{13}C DEPT 135 spectra, 12 of these belonged to protonated C atoms. Of those, the ones at 15.0 and 17.0 were characteristic of two methyls; 35.0, 36.0, and 42.12, three cyclic methylenes; 112.0, an exocyclic methylene; 42.0, 58.0, 72.0, 76.0, and 83.0, five methines; 137.0, an olefinic C atom. Resonances at 138.0, 152.0, and 180.0 ppm in the ^{13}C NMR spectrum were due to three unprotonated C atoms, i.e., C-10, C-4, and C-12, respectively.

Because the resonance of the gem-hydroxyl proton of one OH group overlapped the resonance of the lactone proton (3.84 ppm), the structure of the overlapping resonances could not be examined. Therefore, iskenderolide was acetylated. This produced a compound of formula $C_{19}H_{26}O_6$, mp 142–143°C. Its IR spectrum showed absorption bands for a γ-lactone C=O (1790 cm^{-1}), ester C=O (1730, 1230), and double bonds (1675, 1650). The PMR spectrum exhibited two 3H singlets (2.01, 2.08 ppm) belonging to two acetyl groups. Now the gem-acetyl protons appeared in the spectrum as a doublet with weakly split (~1.5 Hz) components at 4.65 ppm (1H, J = 9.20 Hz) and a triplet at 5.40 ppm (1H, J = 9.65 Hz). The lactone proton appeared as a 1H multiplet at 3.9 ppm. A doublet at 1.35 ppm (3H, J = 7 Hz, CH_3–CH<) and a singlet at 1.70 ppm (3H, CH_3–C=) in the PMR spectrum of diacetyliskenderolide belonged to secondary and vinylmethyl groups, respectively.

Thus, the studied compound contained two secondary hydroxyls.

Assuming that the hydroxyls in iskenderolide were located on neighboring C atoms (C-2 and C-3), we attempted to prepare an acetonide. However, the reaction was negative. In analyzing the reason for the negative reaction, we noticed that the spin—spin coupling constants (SSCC) of the gem-hydroxyl protons of 9.20 and 9.65 Hz did not coincide although they were similar. These constants provided a basis to assign an identical α-orientation to these protons. Therefore, steric hindrance to the formation of the acetonide was not present.

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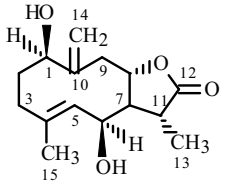
TABLE 1. PMR Spectral Data of Iskenderolide (**1**) and (11*R*)-11,13-Dihydrotatridin-B (**2**), J/Hz

C atom	**1**	**2**
1	3.84 (2H)	4.00 (2H)
5	5.25 (1H, d, $J_{5,6}$ = 9.65)	5.30 (1H, d, $J_{5,6}$ = 10.00)
6	4.35 (1H, t, $J_{6,5}$ = $J_{6,7}$ = 9.65)	4.40 (1H, dt, $J_{6,OH}$ = 4,5; $J_{6,5}$ = $J_{6,7}$ = 10.00)
8	3.84 (2H)	4.00 (2H)
11	2.60 (1H, m)	2.60 (1H, dq, $J_{7,11}$ = 10.00; $J_{11,13}$ = 7.00)
13	1.42 (3H, d, J = 7.00)	1.34 (3H, d, J = 7.00)
14	5.13 (1H, s)	5.01 (1H, s)
	5.40 (1H, s)	5.08 (1H, s)
15	1.60 (3H, s)	1.70 (3H, s)

Protons coupled to each other are known to have identical SSCC [6, 7]. Resonances for the vinylmethyl olefinic protons in PMR spectra of iskenderolide and its diacetyl derivative (5.25 ppm, d, and 5.45 ppm, t, respectively) had SSCC of 9.65 Hz. This constant coincided with that for one of the gem-hydroxyl protons. Therefore, the vinylmethyl group was involved in the formation of a secondary–tertiary double bond, which is traditionally located at C_4–C_5 in germacranolide dienes [7–9].

Judging from this and the presence in the PMR spectrum of a second resonance (besides the resonance of the olefinic proton) at 4.35 ppm with SSCC 9.65 Hz that belonged to one of the gem-hydroxyl protons (H-6), it was concluded that iskenderolide had the structure 1β,6β-dihydroxygermacr-4(5),10(14)-dien-8,12-olide.



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The structure proposed for iskenderolide (**1**) was very similar to that of (11*R*)-11,13-dihydrotatridin-B (**2**), which was isolated from *Chrysanthemum cinerariaefolium* Vis. (Table 1) [10].

Like (11*R*)-11,13-dihydrotatridin-B, **1** contained two secondary hydroxyls on C_1 and C_6. In contrast with **1**, for which both hydroxyls had the β-orientation, one of them in **2**, namely on C_6, had the α-orientation. This was consistent with both the multiplicity and SSCC for H-6 of the compared lactones. The 1H multiplet at 2.60 ppm in the PMR spectrum of **1** consisted of six lines. The chemical shift and structure of the resonance were indicative of the possible α-position of the methyl on the lactone ring.

Thus, consideration of the results obtained from solving NMR spectra of **1** and its diacetate and from a comparison with those of (11*R*)-11,13-dihydrotatridin-B, **1** was assigned the structure C_6-*epi*-(11*R*)-11,13-dihydrotatridin-B.

EXPERIMENTAL

IR spectra were recorded in mineral oil on a UR-20 spectrophotometer. PMR and ^{13}C NMR spectra were taken in $CDCl_3$ on a Bruker 300 spectrometer (300 MHz for ^1H and 75 MHz for ^{13}C). Chemical shifts were determined relative to TMS internal standard on the δ-scale. Melting points were measured on a Boetius stage. The purity of the compounds was established on Silufol UV 254 chromatographic plates.

Isolation of Iskenderolide. Finely ground air-dried aerial part of *A. iskenderiana* Rzazade that was collected in August in Konakhkend District, Azerbaidzhan Republic was extracted with acetone (3×, each time for 3 d). The acetone extracts were filtered and evaporated to afford dark-green (13.0 g) extracted compounds (4.64% yield) that were chromatographed over a column of neutral Al_2O_3 (III-IV Brockmann activity) (h = 100, d = 3.5 cm) with elution by hexane, hexane:$CHCl_3$ (9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8, and 1:9), and $CHCl_3$. The volume of each fraction was 100 mL.

Fractions 64–66 (hexane:$CHCl_3$, 1:1) isolated a crystalline compound of formula $C_{15}H_{22}O_4$, mp 190–191°C (aq. EtOH), yield 2.5% of resin weight. IR spectrum (ν, cm^{-1}): 3300 (OH), 1755 (γ-lactone C=O), 1655, 1650 (C=C).

PMR spectrum (300 MHz, $CDCl_3$, δ, ppm, J/Hz): 1.42 (3H, d, J = 7, CH_3–CH<), 1.60 (3H, s, CH_3–C=), 3.84 (2H, m, H-1, H-8), 4.35 (1H, t, J = 9.65, H-6), 5.13 and 5.40 (1H each, s, H_2C=C<), 5.25 (d, J = 9.65, H-5).

Acetylation of 1. Compound **1** (0.05 g) was dissolved in Py (2 mL), treated with acetic anhydride (2.5 mL), heated on a water bath for 3 h, left overnight at room temperature, transferred to a porcelain crucible, and evaporated on a water bath. The solid was dissolved in $CHCl_3$ (10 mL), filtered through a 10-cm layer of neutral Al_2O_3, and eluted with $CHCl_3$ (50 mL). The $CHCl_3$ was evaporated. The solid was a crystalline compound of formula $C_{19}H_{26}O_6$, mp 142–143°C (hexane:$CHCl_3$). IR spectrum (ν, cm^{-1}): 1790 (γ-lactone C=O), 1730, 1230 (acetyl –CO groups), 1675, 1650 (–C=C–).

PMR spectrum (δ, ppm, J/Hz): 1.35 (3H, d, J = 7, CH_3–CH<), 1.70 (3H, s, CH_3–C=), 3.90 (1H, m, H-8), 4.65 (1H, q, J_1 = 9.20, J_2 = 1.5, H-1), 5.19 and 5.35 (1H each, s, H_2C=C<), 5.21 (d, J = 9.65, H-5), 5.40 (1H, t, J = 9.65, H-6).

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