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Ajanolide A, a new germacranolide from *Ajania fruticulosa*

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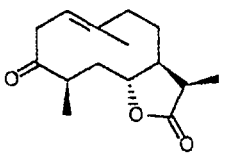
A new germacranolide, ajanolide A, was isolated from aerial parts of *Ajania fruticulosa* by means of extraction with $CHCl_3$ and adsorption chromatography. This compound was identified as (1(10)*E*,3*S*,4*Z*,6*R*,7*S*,11*R*)-3-acetoxygermacra-1(10),4-dien-12,6-olide ((1*S*,7*S*,10*R*,13*R*)-7-acetoxy-4,8,13-trimethyl-11-oxabicyclo[8.3.0]trideca-4(*E*),8(*Z*)-dien-12-one) by X-ray diffraction analysis. 2D $^1H-^1H$ (COSY) and $^{13}C-^1H$ (COSY) NMR spectroscopy was used for assigning the ^1H and ^{13}C NMR signals in the spectra of ajanolide A.

Key words: sesquiterpenoids, germacranolides, X-ray diffraction analysis; two-dimensional NMR spectroscopy.

The botanical genus *Ajania Poljak* (*Asteraceae* family) counts about 25 species.[1] One of these, *Ajania fruticulosa* (Ldb.) *Poljak*, is found in Tien Shan, Pamir-Alay, in Western Siberia, China, and Mongolia. This plant is well known in Chinese folk medicine;[2] for example, the alcoholic extract of its aerial part exhibits myotropic, spasmolytic, vasodilatory, and diuretic properties.[3] The chemical aspects of *A. fruticulosa* have been studied insufficiently. Its aerial part was found to contain three flavonoids[4] and three sesquiterpenic lactones,[5] two of which, the germacranolides ketopelenolide b (**1**) and epitansanine (argolide)[6] (**2**), have been previously isolated from other plants, while guaianolide (**3**) is a new compound.



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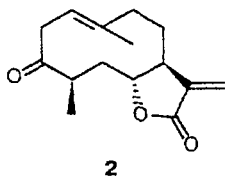

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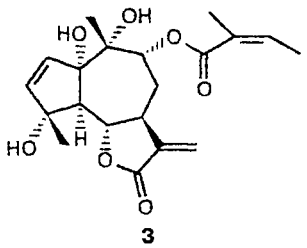

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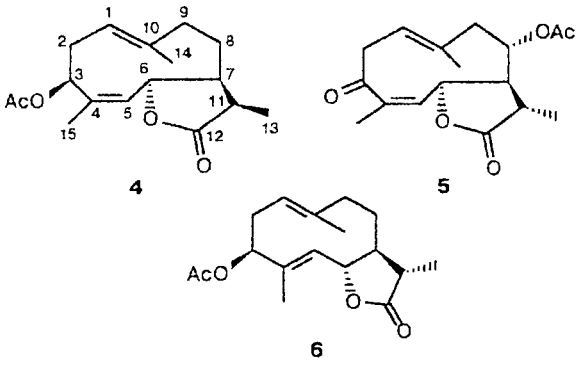
It is known that sesquiterpenic lactones display a broad spectrum of biological activity.[7] It is possibly their presence in the *Ajania fruticulosa* that is responsible for its valuable pharmacological properties. Therefore we carried out an additional phytochemical study of the aerial part of this plant collected in Central Kazakhstan. The lactones were isolated using the general procedure reported previously.[8] This procedure afforded three crystalline lactones, of which the major one (yield 0.02% with respect to the air-dry plant) was found to be a new compound never reported before. This lactone, named ajanolide A, has the molecular formula $C_{17}H_{24}O_4$ (according to data of high resolution mass-spectrometry). The four O atoms in its molecule are involved in the γ-lactone and acetoxy groups (according to the IR spectrum). The ^{1}H NMR spectrum suggests that the molecule of ajanolide A incorporates four Me groups, two of which are located at the double bonds, one is involved in the acetoxy group, while the fourth one is a secondary group.



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The structure of the ajanolide A molecule was established by means of X-ray diffraction analysis (Fig. 1). It corresponds to structure **4**. The bond lengths in molecule **4** are close to the mean statistical values.[9] Its ten-membered cycle has one of the most stable conformations for cyclodecane, referred to as boat-chair-chair.[10] The lactone cycle has the usual[10] twist-form $^7T_{11}$ with a C(6)—C(7)—C(11)—C(12) torsion angle of −30.8(4)°.

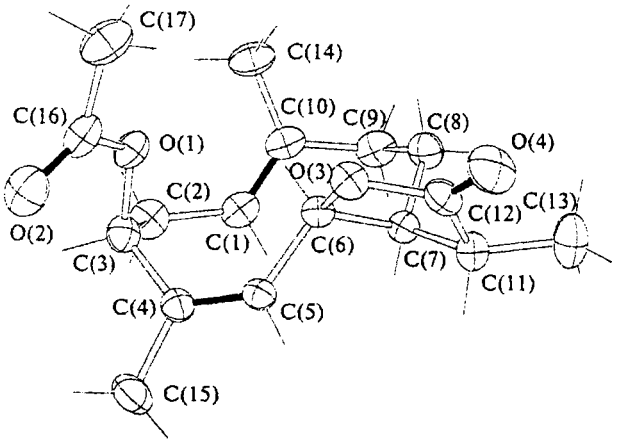


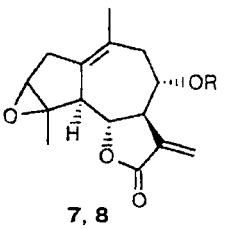
Fig. 1. Structure of molecule **4**.

A search in the Cambridge Bank of structural data[11] revealed information of only one germacra-1(10),4-dienic lactone, heliangolidine (**5**),[12] which was isolated from *Artemisia canariensis* Lees (family *Asteraceae*) and had a structure of the bicyclic frame similar to that of lactone **4**. Despite the differences in the side substituents, the macrocycle conformation in lactone **4** is the same as that in compound **5** (boat-chair-chair), while some difference in the torsion angles is observed only for the C(5)C(6)C(7)C(8)C(9)C(10) moiety. The lactone cycle is planar in molecule **5** but non-planar in the molecule of ajanolide A.

The ^{1}H and ^{13}C NMR spectra of ajanolide A (Table 1) were recorded in C_6D_6 instead of the commonly used $CDCl_3$, because the ^{1}H NMR spectrum recorded in the latter shows a group of overlapping signals of three protons in the region of δ 5.03—5.33. The spectra for the solutions in C_6D_6 were interpreted using two-dimensional ^{13}C—^{1}H NMR (COSY) spectroscopy and ^{1}H—^{1}H NMR (COSY) spectroscopy. Some of the signals in the ^{1}H NMR spectrum are broadened due to the conformational exchange. When the solution is heated to +60 °C, only broadening of the signal from the HC(1) olephinic proton remains (Table 1), which is probably caused by allylic spin-spin interaction.

One should note the small coupling constant $^3J_{H(6),H(7)}$ (1.6 Hz), which is also small for molecules of lactone **5** (Ref. 12) and other (1(10)*E*,4*Z*)-germacradienic lactones (heliangolides).[13] The configuration of the Δ^4-double bond is undoubtedly of primary importance in this case, since $^3J_{H(6),H(7)}$ = 8.5 Hz in the spectrum of the natural stereoisomer of ajanolide A, 3β-acetoxy-11β,13-dihydrocostunolide (**6**).[14]

The second lactone that was isolated from the plant studied (yield 0.001%) proved to be its known component, ketopelenolide b **1**, while the third one (yield 0.002%) was identified as arteglazine A (**7**)[15] according to its ^{1}H NMR spectra. Its 8-*O*-deacyl derivative **8** has been described under the name ajadine and later isolated from the aerial part of *Ajania fastigata (Compositae* family).[16]



7, 8

R = Ac (**7**), H (**8**)

Experimental

Melting points were determined on a Koefler stage. IR spectra were obtained on a UR-20 instrument in CCl_4. NMR spectra were recorded on a Bruker DRX-500 spectrometer (working frequency 500.13 MHz (^{1}H), 125.76 MHz (^{13}C), with $SiMe_4$ as the internal standard), using standard programs provided by Bruker for recording two-dimensional COSY spectra. Mass spectra (EI, 70 eV) were obtained on a Finnigan

Table 1. Parameters of the ^1H and ^{13}C NMR spectra of compound **4** (C_6D_6, [**4**] = 20 mg mL^{-1})

Atom, group	δ ^{13}C (+30 °C)	δ ^1H (*J*/Hz)	
		+30 °C	+60 °C [a]
C(1), H(1)	122.15 (d)	4.83 (br.m)	4.89 (br.t, J = 8)
C(2), H(2)	29.61 (t)	2.42 (br.t, H(2a), J = 12); 1.86 (br.dt, H(2b), J = 14, J = 5)	2.44 (ddd, J = 14.5, J = 9.5, J = 2.5); 1.92 (ddd, J = 14.5, J = 7.0, J = 4.0)
C(3), H(3)	77.21 (d)	5.07 (dd, J = 4.0, J = 2.5)	5.10
C(4)	138.00 (s)	—	—
C(5), H(5)	126.31 (d)	4.96 (dq, J = 11.0, J = 1.4)	5.01
C(6), H(6)	80.84 (d)	5.03 (br.d, J = 10)	5.07 (dd, J = 11.0, J = 1.6)
C(7), H(7)	44.67 (d)	1.43 (ddt, J = 12.0, J = 10.0, J = 1.6)	1.57
C(8), H(8)	24.38 (t)	1.14 (dtd, H(8a), J = 14.0, J = 4.0, J = 1.6); 0.93 (br.t, H(8b), J = 13)	1.23, 1.02 [b]
C(9), H(9)	40.67 (t)	1.95 (br.d, H(9a), J = 13); 1.66 (br.t, H(9b), J = 13)	2.01 (dt, J = 12.5, J = 4); 1.71 (td, J = 12.5, J = 4.0)
C(10)	135.87 (s)	—	—
C(11), H(11)	35.87 (d)	2.33 (dq, J = 10.0, J = 7.5)	2.39
C(12)	178.45 (s)	—	—
C(13)H_3	11.00 (q)	1.01 (d, 3 H, J = 7.5)	1.02
C(14)H_3	16.86 (q)	1.60 (br.s, 3 H)	1.65
C(15)H_3	23.30 (q)	1.58 (d, 3 H, J = 1.4)	1.59
C(16)	168.96 (s)	—	—
C(17)H_3	20.76 (q)	1.76 (s, 3 H)	1.77

[a] The multiplicity and coupling constants are only given for the signals whose pattern changes when the temperature of the specimen is increased to +60 °C.
[b] The signal overlaps that of the C(13)H_3 group.

MAT 8200 instrument. Optical rotation was determined in $CHCl_3$ on a Polamat A polarimeter (at 580 nm).

Column chromatography was performed on KCK grade SiO_2. TLC was done on Silufol plates. A 1% aqueous solution of $KMnO_4$ was used for visualization.

The aerial parts of *Ajania fruticulosa* were collected in 1996 during the budding phase (August) around the Dolagan village (Semipalatinsk Region), dried in the air, and crushed.

Isolation of lactones. A weighed sample (1 kg) of the raw material was placed in a 5 L flask equipped with a reflux condenser, $CHCl_3$ (3.5 L) was added, and the mixture was heated to boiling. After cooling, the extract was removed, and the extraction was repeated two times (2×3.5 L). A similar extraction was conducted with an additional 1 kg of the material. The combined chloroform extracts were filtered, and the solvent was evaporated to dryness. An EtOH—H_2O mixture (2 : 1, v/v; 0.9 L) was added to the dry extract; the mixture was thoroughly stirred, and the precipitate (130 g) was filtered off. The filtrate was successively extracted with petroleum ether (3×0.1 L), benzene (3×0.1 L), and $CHCl_3$ (3×0.1 L). The chloroform extracts were combined, and the solvent was evaporated to dryness. The resulting product (45 g) was chromatographed on SiO_2 (450 g) using benzene and then a benzene—AcOEt mixture as the eluents. The benzene eluates were combined and evaporated to dryness. The residue (5 g) was chromatographed on SiO_2 (250 g). The column was eluted in the gradient petroleum ether—AcOEt (100 : 0 → 70 : 30) to give successively lactone **1** (0.025 g), compound **4** (0.440 g), and lactone **7** (0.040 g).

Arteglazine A (7). M.p. 205—207 °C, $[\alpha]_{580}^{22}$ +133° (*c* 0.96, $CHCl_3$) (lit. data: m.p. 207—208 °C,[15] 202—203 °C;[16] $[\alpha]_D^{25}$ +110° ($CHCl_3$),[15] $[\alpha]_D^{22}$ +112° ($CHCl_3$)[16]). The ^1H NMR spectroscopic parameters agree with those reported previously for arteglazine A[15] and for acetylajadine.[16]

Ajanolide A (4). Crystals, m.p. 125—126 °C (petroleum ether—AcOEt), $[\alpha]_{580}^{22}$ +142.8° (*c* 0.56, $CHCl_3$). IR (CCl_4), ν/cm^{-1}: 1780 (γ-lactone); 1740, 1035 (OAc); 1237, 1220, 1175, 1110, 960, 935, 875. MS, *m/z* (I_{rel} (%)): 292 $[M]^+$ (19), 250 (26), 232 (63), 217 (15), 177 (22), 159 (42), 158 (21), 123 (35), 121 (31), 107 (28), 43 (100). Found: *m/z* 292.16844 $[M^+]$. $C_{17}H_{24}O_4$. Calculated: M = 292.16745. The ^1H and ^{13}C NMR spectra are presented in Table 1.

The X-ray diffraction study of lactone 4 (specimen size 0.7×0.4×0.25 mm) was performed on a Syntex $P2_1$ diffractometer. Crystal parameters: a = 9.037(1) Å, b = 13.084(2) Å, c = 14.158(2) Å, V = 1674.0(4) Å^3, space group $P2_12_12_1$, $C_{17}H_{24}O_4$, M = 292.36, Z = 4, d_{calc} = 1.16 g cm^{-3}, λ = 1.54178 Å (Cu-Kα irradiation, graphite monochromator), μ = 0.659 mm^{-1}. The intensities of 1817 independent reflections with 2θ < 140° were measured using θ/2θ-scanning. Correc-

Table 2. Coordinates ($\times 10^4$) of non-hydrogen atoms and equivalent isotropic thermal parameters ($\times 10^3$) in the structure of lactone **4**

Atom	x/a	y/b	z/c	U_{eq}/Å^2
C(1)	1376(5)	425(3)	6834(3)	76(1)
C(2)	1049(6)	926(3)	5907(3)	87(1)
C(3)	450(5)	2026(3)	6000(3)	72(1)
C(4)	−806(4)	2155(2)	6706(2)	63(1)
C(5)	−642(4)	2298(2)	7626(2)	56(1)
C(6)	755(4)	2260(2)	8195(2)	52(1)
C(7)	746(4)	1407(2)	8951(2)	57(1)
C(8)	2267(5)	916(3)	9081(3)	78(1)
C(9)	2616(5)	98(3)	8345(3)	86(1)
C(10)	2608(5)	485(3)	7344(3)	74(1)
C(11)	162(5)	1991(3)	9811(2)	70(1)
C(12)	723(4)	3057(3)	9650(3)	68(1)
C(13)	461(7)	1578(4)	10797(3)	116(2)
C(14)	3998(5)	1015(3)	7027(4)	96(1)
C(15)	−2309(5)	2100(4)	6265(3)	100(2)
C(16)	1558(5)	3666(3)	6062(3)	77(1)
C(17)	2838(6)	4244(3)	6452(4)	111(2)
O(1)	1700(3)	2663(2)	6245(2)	70(1)
O(2)	543(4)	4022(2)	5649(2)	102(1)
O(3)	946(3)	3214(2)	8725(2)	64(1)
O(4)	924(4)	3718(2)	10219(2)	99(1)

tions for absorption (transmission 0.71—0.86) were introduced analytically, based on the actual crystal faceting. The structure was solved by the direct method, using the SHELXL-86 program, and refined by the full-matrix method in the anisotropic approximation to $wR_2 = 0.1376$ and $S = 1.040$ for all reflections ($R = 0.0458$ for 1359 $F > 4\sigma$) using the SHELXL-93 program. The positions of the hydrogen atoms were recalculated geometrically during the approximation. The resulting coordinates of non-hydrogen atoms are presented in Table 2.

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