## Identification of α-santonin in Artemisia marschalliana Spreng. collected in Azerbaijan

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Abstract: The plants of the genus Artemisia L. are rich in biologically active secondary metabolites. Phytochemical study of secondary metabolites of plants of the genus Artemisia has led to the creation on their basis, in particular, on the sesquiterpene lactones, antitumor (artemisinin and its derivatives; arglabin), antiatherosclerotic (leucomisin (preparation oligvon)), cardiotonic (tauremisin), anthelmintic (α-santonin) and antimalarial (artemisinin and its derivatives) preparations. This article is dedicated to the research of the chemical compounds of aerial parts A. marshalliana Spreng. The aerial parts of A. marshalliana collected for the first time in the vicinity of the Araz River in the Republic of Azerbaijan during the budding period, identified sesquiterpene lactone eudesmanolide structures. The study of the physicochemical properties of the obtained substance was carried out by spectral methods of UV, IR, <sup>1</sup>H, <sup>13</sup>C, <sup>13</sup>C DEPT 135, 90, 45 NMR spectra and comparison of the results with scientific literature data. As a result, the elemental composition of the substance is C<sub>15</sub>H<sub>18</sub>O<sub>2</sub>, m.p. 170-172°C which has been identified as α-santonin. The result can be used in obtaining the substance and its identification, as well as in chemotoxanomic classification of species of the genus Artemisia L.

**Keywords:** chromatography, eudesmanolide, extraction, sesquiterpene lactone, infrared (IR), ultraviolet irradiation (UV), PMR, <sup>13</sup>C NMR, <sup>13</sup>C DEPT 135, 90, 45 spectroscopy

#### INTRODUCTION

The genus *Artemisia* L. (wormwood) belongs to the Compositae family and there are about 500 species in the world. *Artemisia* species have essential oils [Mahmoud

et al., 2021] characterized by monoterpenoids [Julio et al., 2017], sesquiterpene lactones, steroids, flavonoids [Mukhamatkhanova et al., 2020; Bianca et al., 2015; Braulio et al., 2021; Ibrahimova et al., 2019; Devmurari et al., 2010] etc. These substances are biologically active showing antioxidant, cardiotonic, antitumor, anthelmintic, antitumor properties. For the first time in medical practice, santonin, which is a sesquiterpene lactone, was used as an anthelmintic agent in the treatment of ascariasis [Dheeraj et al., 2010; Dheeraj et al., 2021; Jorge et al., 2010].

The genus *Arthemisia* represented with 42 species in the flora of Azerbaijan [Rzazade, 1955; Kariagin, 1952]. The Shovit's wormwood (*Artemisia szovitsiana* (Bess.) A. Grossh.) was proposed for industrial processing in order to obtain santonin by I.A. Damirov, I.K. Golberg and R.K. Aliyev in the mid XX century [Damirov et al., 1957]. In Azerbaijan, *A. splendens* Willd., *A. fragrans* Willd, *A. maritima* Kitag. were biochemically analyzed, mainly sesquiterpene lactones and essential oils were studied [Serkerov, 2005; Serkerov, Aleskerov, 2006].

It should be noted that the total of sesquiterpene lactones "gelenin" was also obtained from the *Inula helenium* L. species which has a more effective effect than santonin in pediatric practice. Anthelmintic properties were also found in carpesilactone from *Carpesium abrotanoides* L., gafrinin from *Geigeria africana* Griessel. Eremantine-sesquiterpene lactone from *Eremanthus elaeagnus* Sch.Bip., is active against the human parasite *Shistosoma mansoni* Sambon [Rybalko, 1978; Serkerov, 2005].

In Azerbaijan, I.A. Damirov et al. [1957] industrial processing in order to obtain santonin recommended Sovich's wormwood (*A. szovitsiana* (Bess.) A. Grossh.). From the aerial part of *A. annua* L., the sesquiterpene lactone artemisinin was obtained, which is used as an effective antimalarial agent in parasitology (Benye et al., 2011; Ye, Jianrong, 2021]. The purpose of this work is to study the sesquiterpene lactone of *A. marschalliana* Spreng. and use the data to reveal the chemotoxonomic legitimacy of this species.

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#### MATERIAL AND METHODS

The aerial part of *Arthemisia marschalliana* collected on July 28, 2018 during the budding period in the Imishli district of the Republic of Azerbaijan was used as material for research. The amount of biologically active was obtained by extraction.

Extaction. Substances from individual sample was extracted by column chromatography. The individuality of the isolated compounds was determined on "Silufol" (AVALIER) UV-254 and silica gel 60 GF254 (Merck, Germany) plates. The plates were developed using iodine vapor, UV lamp light at 254 nm and 365 nm. The melting point was determined on a Boetius microtable.

Optical rotations were measured on a "Rudolph Reasearch Analytical" digital polarimeter (Autopol I model, USA) (λ 589 nm) at 20°C. The UV spectra of the obtained solutions were recorded on a Cary 60 UV-Vis spectrophotometer [Agilent Technologies, USA]. The IR spectra of the individual substances obtained were recorded on a spectrometer [Varian 640, USA]. <sup>1</sup>H NMR were taken on a "Bruker Fourier-300" spectrometer with a resonant frequency of 300 MHz. Solvent was CDCl<sub>3</sub>. Chemical shifts are given on the δ-scale (ppm). Internal standard was (CH<sub>3</sub>)<sub>4</sub>Si. Chemical shifts are calibrated (<sup>1</sup>H δ 7.27; <sup>13</sup>C δ 77.0 ppm,) (Symbols: s-singlet, d-doublet, t-triplet, k-quartet, sk-sextet, m-multiplet, spin-spin coupling constants (SSCC, J.Hz).

Extraction of the biologically active substances.

Finely ground air-dry material of 300.0 g was extracted with acetone three times, each time for 3 days. Before the use, the acetone was filtered and distilled off in a hot water bath. The weight of the obtained dark green resin was 24.6 g and the yield was 8.2 %.

15.0 g of the obtained green resin was dissolved in 50 ml of chloroform and chromatographed on a column of  $Al_2O_3$  neutral II degree of activity (h=120, d=3.5 cm) was eluted with hexane (30 fraction); a mixture of hexane/benzene in the ratio 8:2, 7:3, 6:4, 5:4, 1:1 (50 fraction); benzene (20 fraction) [Kerimli et al., 2022].

The volume of each fraction was 100 ml. From fractions 60-90, eluted with a mixture of hexane + benzene (1:1), recrystallization from an alcohol-water ratio (5:1) gave a white crystallic substance with the elemental composition  $C_{15}H_{18}O_3$ , m.p. 170-172°C. The UV spectrum of the substance was recorded in ethanol diluted  $2.5 \times 10\text{-}7$  with absolute alcohol. The angle of rotation and the specific index of rotation were determined on a digital polarimeter. Substances in 0.1% chloroform solution (with 0.1; CHCl<sub>3</sub>) [ $\alpha$ ]=-0.27°, [ $\alpha$ 20D]=-270°.

#### RESULTS AND DISCUSSION

As a result of chromatographic separation of the sum of extractive substances, 1 compound was isolated. Based on the spectral data, it was determined that the compound belongs to the sesquiterpene lactone with the eudesmanolide structure and was identified as santonin.

During chromatographic separation, from fraction 60-90 isolated a crystallinec substance of the composition  $C_{15}H_{18}O_3$ , m.p. 170-172°C. In the range of characteristic frequencies of the IR spectrum (Fig. 1.) were found ( $v_{max}$  cm<sup>-1</sup>), absorption bands of the carbonyl group 1783 (C=O of the  $\gamma$ -lactone ring), 1658 (C=O of cyclohexenone) and 1632 (conjugated double bond) [Serkerov et al., 2006].

The <sup>1</sup>H NMR spectrum of the studied compound

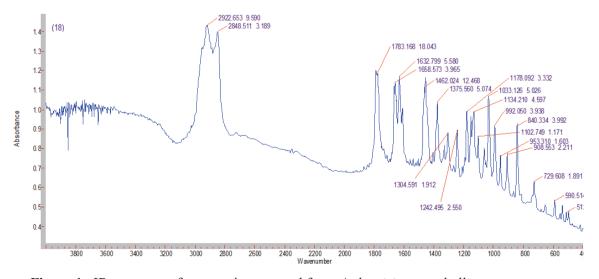


Figure 1. IR spectrum of a santonin extracted from Arthemisia marschalliana.

has a signal a doublet of the secondary methyl group centered at (1.25 ppm, d, J=6.00 Hz, <sup>3</sup>H, CH<sub>3</sub>-CH<, H-13), a singlet of the vinylmethyl group (2.11 ppm. s, 3H, CH<sub>3</sub>-C=, H-15) and a singlet of the angular methyl group (1.31 ppm, <sup>3</sup>H, CH<sub>3</sub>-C, H-14). The proton of the lactone ring is found in the spectrum as a one-proton doublet at 4.77 ppm. (J=11Hz, H-6). The value of the spin-spin coupling constant (J=11 Hz) indicates that the lactone ring is in the trans position and coupling vicinally with the proton in (H-7).

The configuration of the CH<sub>3</sub> group at C-11 was determined on the basis of the value of the vicinal SSCC of the H-11 signal in the PMR spectrum of the substance, which was found in the form of a doublet and a quartet (J<sup>3</sup><sub>H-11, H-13</sub>=7, J<sup>3</sup><sub>H-11, H-7</sub>=11.5 Hz). These data point to the α-orientation of the CH<sub>3</sub> group at C-11. The olefinic proton cycle signals appear as two doublets, each with an area of <sup>1</sup>H at 6.70 ppm. (J=9.9 Hz, H-1) and 6.24 ppm (J=9.9 Hz, H-2). The SSCC of the olefinic protons of the disubstituted double bond is 9.9 Hz [Kerimli et al., 2022] (Fig. 2).

<sup>13</sup>C NMR spectrum, taken with complete suppression of spin-spin interaction with protons, reveals 15 singlet signals (10.90; 12.49; 23.04; 25.13; 37.83, 40.98; 41.37; 53.53; 81.39; 125.84; 128.65; 186.32) which corresponds to the number of carbon atoms in the elemental composition of the compound (Fig. 3).

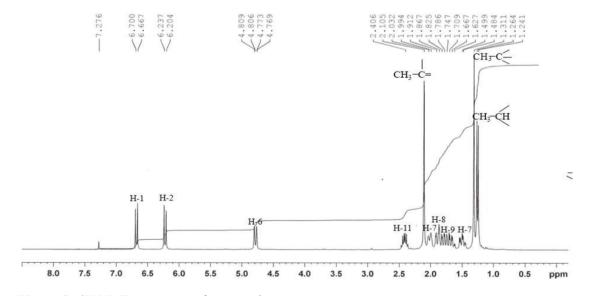
In the <sup>13</sup>C DEPT 45 spectrum found 10 signals (10.90; 12.49; 23.04; 25.13; 37.83; 41.37; 53.53; 81.39; 125.84; 154.97) protonated carbon atoms (Fig. 4a). From these signals in the spectrum of <sup>13</sup>C DEPT 90, 5 protonated carbon atoms of the methine group are

detected, 3 of them without double bonds 41.37; 53.53; 81.39 methine (-CH) and 2 double bond 125.84; 154.97 found in a weak magnetic field refers to methine (-CH=) olefinic groups (Fig. 4b).

<sup>13</sup>C DEPT 135 spectrum in a weak magnetic field reveals 3 methyl (-CH<sub>3</sub>) 10.90; 12.49; 25.13; two methylene (-CH<sub>2</sub>) 23.04; 37.83; five double bonds and no methine-new group bonds (Fig. 4c).

The signals present in the 13C spectrum and not found in the <sup>13</sup>C DEPT 45, 90, 135 spectrum are assigned to unprotonated carbon atoms: (40.98 (>C<) quaternary carbon atom; 128.65 and 151.07 (2>C=); 178.00 carbonyl lactone (-C=O); 186.32 ppm) ketone cyclohexenone. The presence of an angular methyl group in the NMR spectrum indicates that the compound is a lactone belonging to the eudesmanolide group. In the spectrum of <sup>1</sup>H and <sup>13</sup>C DEPT 90, <sup>13</sup>C DEPT 135, it can be seen that the structure of the compound contains 2 olefinic methine (CH=) groups with double bonds. When discussing the location of the olefinic proton in the compound, they can be attributed to carbon atoms C<sub>1</sub>- $C_2$ ,  $C_2$ - $C_3$ ,  $C_8$ - $C_9$ . Then the double bonds can be located  $C_1-C_2:C_4-C_5$ ;  $C_2-C_3:C_4-C_5$ ;  $C_4-C_5:C_8-C_9$ . Based on the spectral data, the following fragment (A, B, C, D, E) structures were assigned to the compound under study (Fig. 5).

If the olefinic protons of the disubstituted double bond are located in position  $C_8$ - $C_9$ , then the proton of the carbon atom at C-8 during vicinal interaction with H-7, H-9, whose areas are 1 proton each gives a triplet, while the proton of the carbon atom at C-9 during vicinal interaction with the H-8 proton of area 1H



**Figure 2.** <sup>1</sup>H NMR spectrum of a santonin.

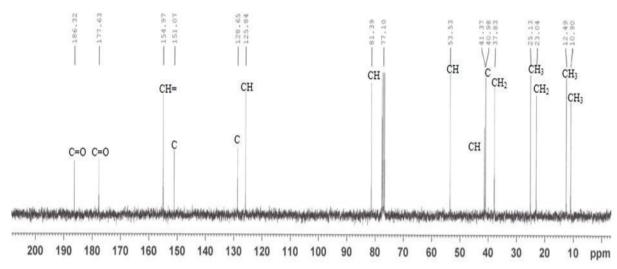
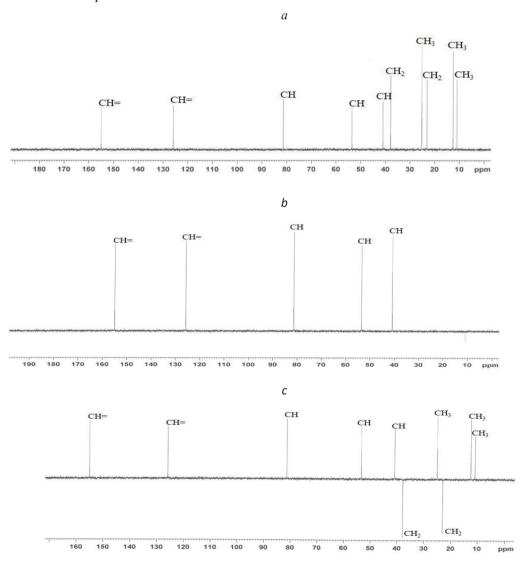


Figure 3. <sup>13</sup>C NMR spectrum of a santonin.



**Figure 4**. <sup>13</sup>C DEPT 45 (a), <sup>13</sup>C DEPT 90 (b), <sup>13</sup>C DEPT 135 (c) NMR spectrum of a santonin.

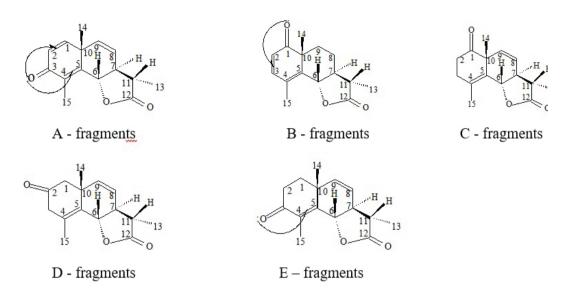


Figure 5. <sup>13</sup>C DEPT 45 (a), <sup>13</sup>C DEPT 90 (b), <sup>13</sup>C DEPT 135 (c) NMR spectrum of a santonin.

should give a doublet. However, this no interpretation of the PMR spectrum. However, this interpretation in the PMR spectrum is missing. Thus, methine groups with a double bond can be located at carbons  $C_1$ - $C_2$ ,  $C_2$ - $C_3$ . It should be noted that the maximum determined in the UV spectrum (EtOH,  $\lambda_{max}$ =238 nm) characterizes the homoannular diene of double bonds in cis-conjugation with the keto group in the same cycle [Fiser et al., 1964; Suchý et al., 1964; Serkerov et al., 2015]. Based interpretation on these information shown C, D and E fragments do not feet to the structure (Fig. 6).

If the keto group of cyclohexenone were conjugated with one double bond, then it would be in position C-1, and double bonds at  $C_2$ - $C_3$  and  $C_4$ - $C_5$ , in this case, an

absorption band 1700 cm<sup>-1</sup> and was higher, however this absorption region is absent in the studied IR spectrum. The detected absorption region of the IR spectrum at 1658 cm-1 indicates that the keto group of cyclohexenone is conjugated by two double bonds in positions  $C_1$ - $C_2$  and  $C_4$ - $C_5$ . This confirms the structure of the connection, in which the ketone group is at C-3. Thus, the structure with fragment A is a more accurate structure, and the compound was named 3-keto-6 $\beta$ ,7 $\alpha$ ,11 $\beta$ H-eudesm-1(2),4(5)-diene-6,12-olide. This is a well-known substance,  $\alpha$ -santonin.

As a result of the conducted research from the aerial part of *Artemisia marschalliana* Spreng. first isolated sesquiterpene lactones eudesmanolide structure

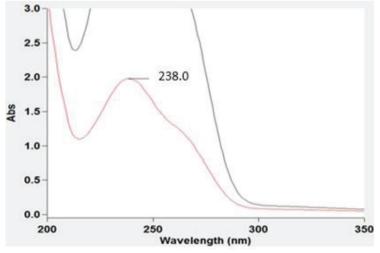


Figure 6. UV- spectrum of a santonin.

composition  $C_{15}H_{18}O_3$  m.p. 170-172°C. Based on the chemical and spectral UV, IR, <sup>1</sup>H, <sup>13</sup>C, <sup>13</sup>C DEPT 45, <sup>13</sup>C DEPT 90, <sup>13</sup>C DEPT 135 NMR spectra of the data, it was proved that this substance has a structure identical to  $\alpha$ -santonin. The result can be used in obtaining the substance and its identification, as well as in chemotoxanomic classification of species of the genus *Artemisia* L.

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# Azərbaycandan toplanan *Artemisia marschalliana* Spreng-dən α-santoninin identifikasiyası

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Artemisia L. cinsinə aid bitkilər bioloji aktiv ikincil metabolitlərin zəngin mənbəyidir. Artemisia cinsinə aid bitkilərin ikincil metabolitlərinin fitokimyəvi tədqiqi onların əsasında, xüsusən seskviterpen laktonların, şişəleyhinə (artemisinin və onun törəmələri; arqlabin), ateroskleroz əleyhinə (leykomizin (Oliqvon preparatı)), kardiotonik (tauremizin), qurdəleyhinə (α-santonin) və malyariya əleyhinə (artemisinin və onun törəmələri) dərmanların yaranmasına səbəb oldu. Tədqiqat isi A. marschalliana Spreng. yerüstü hissəsinin kimyəvi tərkibinin tədqiqinə həsr edilmişdir. Azərbaycanda ilk dəfə olaraq Araz çayının ətrafından yığılmış A. marschalliana növünün butonizasiya mərhələsində yerüstü hissəsindən evdesmanolid qurluşlu seskviterpen lakton izolə olunaraq identifikasiya edilmisdir. Maddənin fiziki-kimyəvi xassələrinin öyrənilməsi spektral UV, IR, <sup>1</sup>H, <sup>13</sup>C, <sup>13</sup>C Dept 45, <sup>13</sup>C DEPT 90, <sup>13</sup>C DEPT 135 NMR üsullarla və onların nəticələrinin elmi ədəbiyyat məlumatları ilə müqayisəsinə əsasən aparılmışdır. Nəticədə maddə element tərkibi C. H. O., ə.t. 170-172°C. olan α-santonin kimi identifikasiya edilmişdir. Nəticələr həm maddənin substansiyasının

əldə edilməsində, onun identifikasiyasında, eləcədə *Artemisia* L. cinsinin növlərinin xemotoksanomik təsnifatında istifadə oluna bilər.

**Açar sözlər:** xromotoqrafiya, evdesmanolid, ekstraksiya, seskviterpen lakton, infraqırmızı (-İQ), ultrabənövşəyi (-UB), PMR, <sup>13</sup>CNMR, <sup>13</sup>CDEPT 135, 90, 45 spektroskopiya

# Идентификация α-сантонина из Artemisia marschalliana Spreng. собраного в Азербайджане

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Растения рода Artemisia L. являются богатыми источниками биологически активных вторичных метаболитов. Фитохимические исследования вторичных метаболитов растений рода Artemisia привели к созданию на их основе, в частности, сесквитерпеновых лактонов, противоопухолевых (артемизинин и его производные; арглабин), антиатеросклеротических (леукомизин (препарат Олигвон)), кардиотонических (тауремизин), антигельминтных (α -сантонин) и противомалярийных (артемизинин и его производные) препаратов. Научная работа посвещена изучению химического состава надземной части A. marshalliana Spreng. Из надземных частей A. marshalliana собранных в первые в окрестностях реки Аракс Азербайджанской Республики в период бутонизации, выделены и идентифицированы сесквитерпеновые лактоны эвдесманолидной структуры. Изучение физико-химических свойств полученного вещества, проводились спектральными методами УФ-, ИК-, 1H, 13C, 13C DEPT 135, 90, 45 ЯМР-спектров и сравнением ее результатов с данными научной литературы. В результате элементный состав вещества С15Н18О3, т.пл. 170-172°С был идентифицирован как а-сантонин. Результаты могут быть использованы при получении субстанции и ее идентификации, а также при хемотоксаномической классификации видов рода Artemisia L.

**Ключевые слова:** хроматография, эвдесманолид, экстракция, сесквитерпеновы лактон, инфракрасная (-ИК), ультрафиолетовая (-УФ), ПМР, ЯМР 13C, 13C DEPT 135, 90, 45 спектроскопия