cally. For this purpose, 1 ml of the extract obtained was mixed in a 50-ml measuring flask with 4 ml of a 0.3% solution of caustic soda, the volume of the solution was made up to 50 ml with water, and the optical density was determined on a SF-4A spectrophotometer at 410 nm in a cell with a layer thickness of 30 mm. In the case of a high optical density, the solution was diluted with water. A standard solution of rutin was prepared by dissolving 0.025 g of rutin in a 100-ml measuring flask in 96% ethanol. The solution obtained (10 ml) was transferred to a 50-ml measuring flask, 4 mole of 0.03% aqueous caustic soda was added, and the solution was made up to the mark and then the optical density of the resulting solution was determined at 410 nm in cell with a layer thickness of 30 mm.

CONCLUSIONS

It has been established that the greatest amount of rutin can be extracted from the eipgeal part of the plant by using 70% ethanol and a degree of grinding of the raw material material of 3 mm. Regression equations have been derived which permit the time necessary for extracting a given amount of rutin to be determined theoretically.

LITERATURE CITED

1. V. D. Ponomarev, The Extraction of Medicinal Raw Material [in Russian], Moscow (1976).
2. T. A. Geissman, in: Biochemical Methods of Plant Analysis (ed. M. N. Zaprometov) [in Russian], Moscow (1960), p. 453.
3. T. Seshadri, in: The Chemistry of Flavonoid Compounds (ed. T. A. Geissman), Pergamon, Oxford (1962).
4. M. D. Mashkovskii, Drugs [in Russian], Mocow (1984).
5. L. V. Ligai, in: Science and Health. Abstracts of Lectures at the IXth Scientific Conference of Young Scientists and Specialists Devoted to the XXVIIth Congress of CPSU [in Russian], Pyatigorsk (1985), p. 27.
6. K. M. Pozin and I. M. Batuner, Mathematical Methods in Chemical Technology [in Russian], Moscow (1963).
7. State Pharmacopoeia of the USSR [in Russian], 10th Edition, Moscow (1968).
8. E. P. Kemertelidze and V. P. Georgievskii Chemical Methods of Analyzing Some Biologically Active Substances of Plant Origin [in Russian], Tbilisi (1977).

ARTENOLIDE — A NEW DISESQUITERPENOID FROM *Artemisia absinthium*

A. Ovezdurdyev, N. D. Abdullaev, M. I. Yusupov, and Sh. Z. Kasymov*

UDC 547.314+543.422+541.67

A new sesquiterpene lactone, artenolide — $C_{30}H_{40}O_8$, mp 163-174°C (ethanol) — has been isolated from the epigeal part of *Artemesia absinthium* L. On the basis of an analysis of spectral characteristics (IR, mass, and ^1H and ^{13}C NMR spectra) of the lactone itself and of a transformation product, a diguaiane structure is suggested for artenolide.

The disesquiterpenoids absinthin [1], anabasin [2], and absintholide [3] have been isolated from *Artemesia absinthium* (common wormwood), family Asteraceae (Compositeae). In the present paper we give information on the isolation and a proof of the structure of a new disesquiterpenoid from common wormwood growing in the Tashkent province — artenolide (I) with the composition $C_{30}H_{40}O_8$, mp 163-174°C (ethanol).

The mass spectrum of (I) did not contain the peak of the molecular ion, but there was a peak of an ion with m/z 510 (M — 18). A broad band at 3350-3500 cm^{-1} in the IR spectrum of (I) showed the presence of hydroxy groups, while bands at 1752-1768 cm^{-1} were due to γ-

*Deceased.

Institute of Chemistry of Plant Substances of the Uzbek SSR Academy of Sciences, Tashkent.

Translated from Khimiya Prirodnykh Soedinenii, No. 5, pp. 667-671, September- October, 1987. Original article submitted February 9, 1987.

TABLE 1. Chemical Shifts of the Signals of the Carbon Atoms in the ^{13}C NMR Spectrum of Artenolide (C_5D_5N, O—TMS, δ)

C atom	ppm	C atom	ppm	C atom	ppm	C atom	ppm
1	153,6	9	39,4	1′	147,9	9′	39,3
2	128,9	10	69,4	2′	52,1	10′	71,7
3	79,7	11	60,0	3′	53,3	11′	42,6
4	87,5	12	180,5	4′	56,7	12′	178,7
5	60,6	13	35,1	5′	143,5	13′	13,0
6	82,3	14	27,5	6′	83,9	14′	30,5
7	43,2	15	17,3	7′	59,2	15′	17,3
8	21,5			8′	25,5		

lactone carbonyl groups and a band at 1645 cm^{-1} to double bonds.

Four hydroxyl groups in the artenolide molecule were revealed by a study of its PMR spectrum. The signals of the hydroxy groups were represented in the form of three singlets at 5.53, 5.88, and 6.21 ppm and a doublet at 7.02 ppm with ^3J = 4.7 Hz. This was confirmed by the fact that when the temperature of the sample was raised these signals shifted in the upfield direction. The multiplicities of the signals indicated that the arternolide molecule contained three tertiary and one secondary hydroxy groups. A broadened signal at 5.28 ppm with an intensity of 1 H and a half-width of 9 Hz in the PMR spectrum of (I), corresponding to a proton located in the geminal position to a hydroxy group, also confirmed the presence of a secondary hydroxy group.

When artenolide was acetylated with acetic anhydride in pyridine, the monoacetyl derivative (II) was obtained, in the spectrum of which the signal of the proton in a geminal position to the acetyl group appeared in a weaker field, at 6.1 ppm.

In the PMR spectrum of (I) there were the signals of two lactam protons: a doublet of doublets at 4.87 ppm with Σ^3J = 20.9 Hz and a doublet at 6.02 ppm with ^3J = 10.0 Hz, while in the ^{13}C NMR spectrum there were two singlets at 178.4 and 180.5 ppm, corresponding to the carbon atoms of γ-lactone C=O groups, i.e., the artenolide molecule contains two γ-lactone rings.

Thus, the functions of the eight oxygen atoms had been found: three tertiary and one secondary hydroxy groups, and two γ-lactone rings.

In the ^{13}C NMR spectrum of (I) there were the signals of five methyl groups in the form of quartets at 13.0, 17.3, 17.3, 27.5, and 30.5 ppm. In the PMR spectrum of (I) the signals of the methyl groups were represented in the form of four singlets at 1.65, 1.61, 1.60, and 1.60 ppm, and a doublet at 1.21 ppm, with J = 6.4 Hz. The multiplicities of these signals indicated the presence in the artenolide molecule of four tertiary and one secondary methyl groups. The values of the chemical shifts of these signals showed that three of the methyl groups were in geminal positions in relation to hydroxy groups.

The dehydrogenation of artenolide in the presence of selenium gave chamazulene.

Thus, artenolide consists of two sesquiterpene lactones linked to one another. The absence of a sixth methyl group in the molecule of (I) showed that the guaiane fragment A is linked to the other sequiterpene moiety through a methylene group, as in the handelin molecule [4].

The PMR spectrum of artenolide had a single signal of an olefinic proton in the form of a doublet of doublets at 6.21 ppm with H = 2.5 and 1.5 Hz.

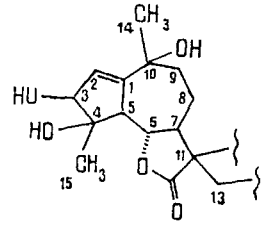
When the olefinic proton was irradiated with an additional radiofrequency field, two signals changed simultaneously: the broadened singlet at 5.28 ppm and the doublet of triplets at 3.29 ppm. When the hydrogen atom at the secondary hydroxy group was irradiated (5.28 ppm), the signals of the olefinic proton simplified to a doublet with G = 2.5 Hz, and the doublet of triplets at 3.29 ppm formed a quartet with J = 10.9 and 2.5 Hz. These facts indicate the mutually vicinal positions of the olefinic proton and the proton of the secondary hydroxy group in a cyclopentane ring, and also the long-range spin-spin interaction of H-5 with H-2 and H-3.

Continuing the double-resonance experiments, it was established that when an additional radiofrequency field was imposed on the doublet of triplets at 3.29 ppm the signal of the

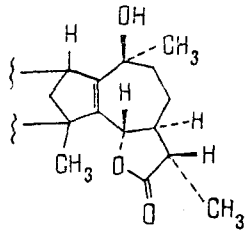
lactone proton — a doublet of doublets at 4.78 ppm — simplified to a doublet with J = 10.0 Hz.

In the selective triple resonance regime with the simultaneous irradiation by radiofrequency fields of the two protons to which the doublet of triplets at 3.29 and the multiplet at 2.49 ppm corresponded, the doublet of doublets at 4.87 ppm simplified to a singlet. When, conversely, a radiofrequency field was imposed at 4.87 ppm, the signals at 3.29 and 2.49 ppm changed into a triplet with ΣJ = 4.7 Hz and a doublet of doublets at J = 12 and 4 Hz.

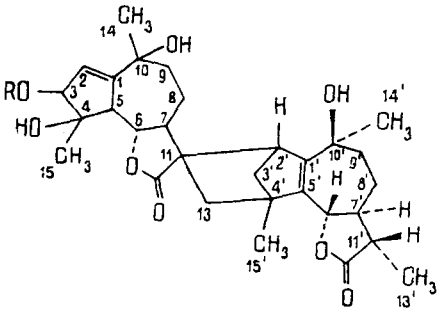
Thus, through the H-5 atom, playing the role of a connecting link, six spin-spin interactions of the protons from H-2 to H-6 of one guaiane fragment of artenolide were traced, and on the basis of the combination of results we suggest structure A.



A



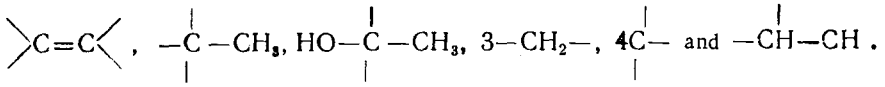
B



I R = H

II R = Ac

The composition $C_{15}H_{20}O_3$ corresponds to the other sesquiterpene fragment. On considering the ^1H and ^{13}C NMR results for artenolide, it was clear that the second half of its molecule containing the following groupings:



These facts showed that the other half of artenolide likewise has the guaiane structure, the signal of the second lactone proton in the PMR spectrum of I being represented in the form of a doublet with ^3J = 10 Hz. The chemical shift of the lactone proton showed that a tetrasubstituted double bond at C=1' and C-5' is presented in the α-position with respect to the lactone proton.

When the protons of the secondary methyl group, giving a doublet at 1.21 ppm were irradiated with an additional radiofrequency field, the multiplet at 2.23 ppm simplified to a doublet with ^3J = 11.8 Hz. This showed the mutual trans orientation of the C-11' and C-7' protons.

The facts presented above permit structure B to be suggested for the second half of artenolide.

The mutual linkage of the two guaiane components A and B through C-11 and C-13, as shown in structural formula (I), was determined on the basis of the presence in the ^{13}C NMR spectrum of arternolide of the signals of a methylene carbon atom and of a spirocarbon atom at 35.1 and 60.0 ppm.

EXPERIMENTAL

IR spectra were taken on a UR-20 instrument (tablets with KBr), mass spectra on a MKh-1310 instrument, PMR spectra on a SC-300 spectrometer, and ^{13}C NMR spectra on a Varian CRT-20 instrument.

R_f values were obtained on Silufol plates in the chloroform–acetone (3:7) system with a 0.5% solution of vanillin in concentrated sulfuric acid as the revealing agent.

Artenolide (I). Leaves and small stems of *Artemisia absinthium* (80 kg) were extracted with ethanol. The concentrated extract was treated with 20% aqueous ethanol. After a day, the precipitate was separated off and the solution was concentrated. The concentrated polar material was dissolved in 3 liters of ethanol, and 9 liters of water containing 250 g of lead diacetate was added. After a day, the precipitate was separated off and the solution was concentrated to 3 liters and was extracted with ethyl acetate 14 times. The aqueous solution was concentrated to 0.6 liter. Then it was again extracted with ethyl acetate, eight times. The ethyl acetate extracts were combined. The elimination of the solvent yielded 350 g of combined polar lactones, which were chromatographed on silica gel of the KSK type (in a ratio of 1:10) with elution successively by benzene, benzene–chloroform (4:1 and 1:1), chloroform, and chloroform–acetate (19:1, 9:1, 4:1, 7:3, 3:2, and 1:1). This gave 0.4 g of (I) (fractions 131-139; chloroform–acetone (7:3) eluate) with the composition $C_{30}H_{40}O_8$, R_f 0.2.

PMR spectrum (δ, ppm, C_5D_5H, O — TMS): 6.21 (doublet of doublets) (J = 2.5 and 1.6 Hz, H-2); 5.28 (broadened singlet, $W_{1/2}$ = 9 Hz, H = 3'); 3.29 (doublet of triplets, J = 10.9, 2.5, and 2.2 Hz, H-5); 4.87 (doublet of doublets, J = 20.9 Hz, H-6); 2.49 (multiplet, H-7); 6.02 (doublet, J - 10.0 Hz, H-6); 1.21 (doublet, J = 6.4 Hz, CH_3-13); 1.60 × 2, 1.61, and 1.85 ppm (singlets, tertiary methyl groups).

Chamazulene. When 50 mg of (I) was heated with an equal amount of amorphous selenium at 250°C for 5 min, a dark blue liquid hydrocarbon was obtained which, after purification on a column of alumina, was identified by its R_f value (in petroleum ether) and its coloration in comparison with an authentic sample.

Artenolide Acetate (II). A solution of 60 mg of artenolide in 1 ml of pyridine was treated with 0.5 ml of acetic anhydride, and the mixture was kept at room temperature for 2 h. The solvent was evaporated off in vacuum and the product was purified by chromatography on type KSK silica gel. It was impossible to crystallized artenolide acetate (II). R_f 0.28.

CONCLUSIONS

A new sesquiterpene lactone — artenolide — has been isolated from the epigeal part of *Artemisia absinthium*. On the basis of an analysis of the spectral characteristics of the lactone itself and a product of its transformation, a diguaiane structure has been suggested for artenolide.

LITERATURE CITED

1. V. Herout, L. Navotny, and F. Sorm, Collect. Czech. Chem. Commun., 21, 1485 (1956).
2. Sh. Z. Kasymov, N. D. Abdullaev, G. P. Sidyakin, and M. R. Yagudaev, Khim. Prir. Soedin., 495 (1979).
3. J. Beauhaire, J. L. Fourrey, and R. Guittet, Tetrahedron Lett., 25, No. 26, 2751 (1984).
4. V. A. Tarasov, N. D. Abdullaev, Sh. Z. Kasymov, G. P. Sidyakin, and M. R. Yagudaev, Khim. Prir. Soedin., 745 (1976).