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ANABSIN — A NEW DIGUAIANOLIDE FROM *Artemisia absinthium**

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UDC 547.314

The new diguaianolide anabsin, $C_{30}H_{40}O_7$, mp 276°C (decomp.), $[\alpha]^{25}$ +110° (c 1.7; acetone) has been isolated from *Artemisia absinthium* L. Anabsin acetate, dehydroanabsin, and dehydroanabsin acetate have been obtained. A comparative study of the ^1H and ^{13}C NMR spectra of anabsin, absinthin, and anabsinthin has been made. The structure of anabsin has been established and structures have been suggested for absinthin and anabsinthin. The most probable biogenesis of anabsin in plants has been put forward.

Continuing a study of the sequiterpene lactones of *Artemisia absinthium* L., family Compositae, in addition to the known lactones artabsin and absinthin [1, 2], we have isolated a new compound anabsin (I), $C_{30}H_{40}O_7$, mp 276°C (decomp.), $[\alpha]_D^{25}$ +110° (c 1.7; acetone).

Anabsin dissolves on heating in dilute solutions of alkalis, and on acidification is liberated in unchanged form, which is characteristic for compounds containing a lactone ring.

The IR spectrum of (I) (Fig. 1) has absorption bands at 3385 and 1760-1780 cm^{-1}, which are characteristic for a hydroxy group and the carbonyl of a γ-lactone ring. The signals of the carbon atoms of the carbonyls of the ester groups in the ^{13}C NMR spectrum appear in the form of singlets at 178.4 and 178.9 ppm. Consequently, in the anabsin molecule four oxygen atoms participate in the formation of two γ-lactone rings.

The PMR spectrum of (I) (Fig. 2) shows the three-portion signals of six methyl groups: doublets at 0.97 and 1.09 ppm with ^3J = 6.7 Hz, 2 (CH—CH_3); singlets at 1.22, 1.31, and 1.57 ppm, 3 (—C—CH_3); broadened singlet at 1.90 ppm (—CH=C—CH_3). The signals of protons geminal to lactone oxygen atoms (lactone protons) appear in the form of one-proton doublets at 4.18 and 4.74 ppm with ^3J = 9.7 and 9.0 Hz, respectively, the components of the second doublet being considerably broadened. The nature of the splitting of these signals shows that each of the lactone protons interacts with only one vicinal proton. A broadened one-proton singlet at 4.07 ppm with $J \lesssim 1$ Hz belongs to a proton in the geminal position to a secondary hydroxy group (hemihydroxylic proton) of the molecule of (I), since it is just the signal that is shifted downfield and is present at 5.07 ppm in the spectrum of the acetyl derivative of anabsin (II). Anabsin acetate was obtained by the acetylation of (I) with acetic anhydride in pyridine. The signals of three methine protons had the form of broadened doublets at 2.81 and 3.77 ppm with $^3J = {}^3J$ = 9.9 Hz and a singlet at 2.69 ppm, and the

*Delivered at the Eleventh International Symposium on the Chemistry of Natural Compounds, Bulgaria, September, 1978 [7].

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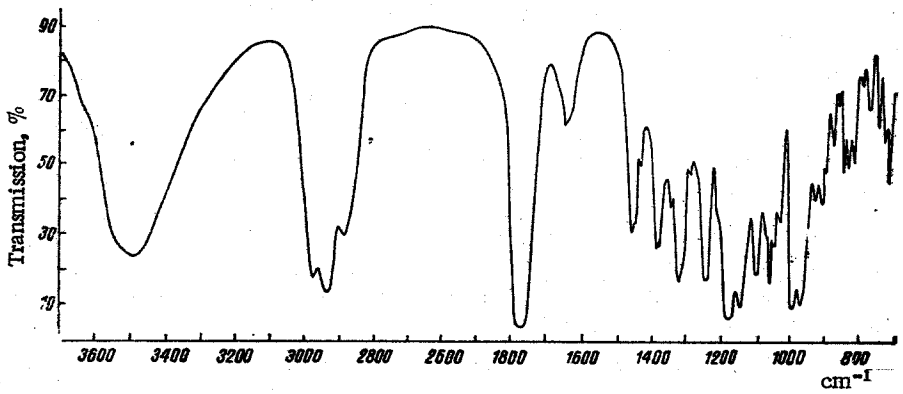


Fig. 1. IR spectrum of anabsin.

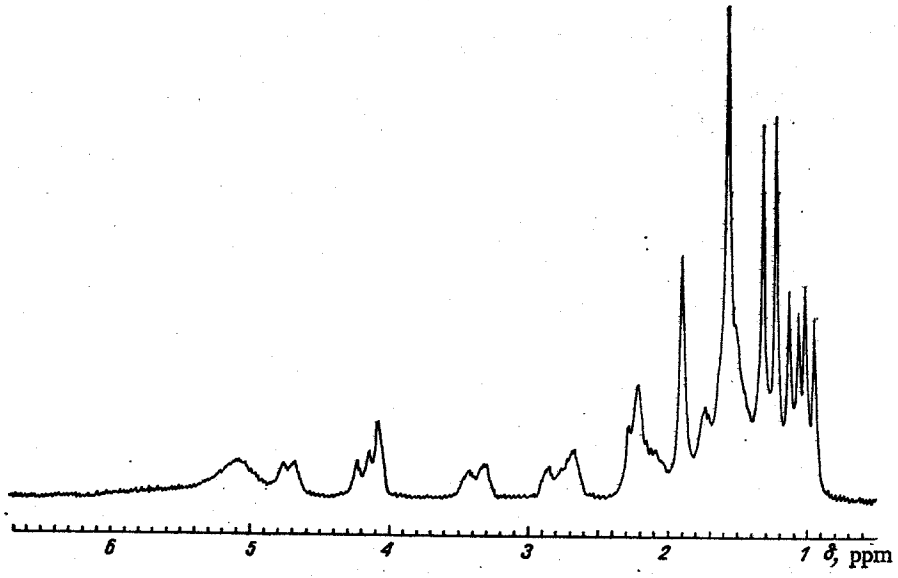


Fig. 2. PMR spectrum of anabsin.

signals of the other methine and methylene protons overlapped one another.

The oxidation of anabsin with chromium trioxide in pyridine led to dehydroanabsin (III). In the IR spectrum of (III) the absorption band due to the stretching vibrations of the carbonyl group formed fused with the absorption band of the C=O groups of the γ-lactone rings and was represented by a maximum at 1750-1770 cm^{-1}.

The PMR spectrum of dehydroanabsin, unlike that of the initial lactone, lacked the signal of a hemihydroxylic proton.

The acetylation of (III) with acetyl chloride gave dehydroanabsin acetate (IV). The IR spectrum of (IV) lacked the absorption band of a hydroxy group and had maxima at 1765 and 1780 cm^{-1} (C=O of γ-lactone rings), 1750 cm^{-1} (C=O in a cyclopentane ring), and 1735 and 1250 cm^{-1} (O–CO–CH_3). It follows from this that the anabsin molecule includes two hydroxy groups — secondary (in a five-membered ring) and tertiary.

Thus, the functions of six of the oxygen atoms of the anabsin molecule have been determined. The seventh oxygen atom obviously participates in the formation of an ether bridge. Anabsin is not reduced in acetic acid in the presence of platinum oxide, although there is a double bond in its molecule.

As mentioned above, anabsin was isolated together with absinthin (VI), and their compositions differ only by one oxygen atom.

Absinthin was first isolated by V. Herout et al. [3]. They suggested for it a diguaiane structure [4]. The diguaiane nature of absinthin was later confirmed by the formation of two guaianolides when it was heated in decalin [5].

It is known that in a weakly acidic medium absinthin isomerizes into anabsinthin (V) [4].

The PMR spectrum of (V) has a complex of signals formed by the mutual superposition of two doublets and two singlets with their center at 1.11 ppm [2 (CH—CH_3) and 2 (C—CH_3)], a singlet at 1.36 ppm (HO—C—CH_3), and a broadened singlet at 1.93 ppm (C=C—CH_3). Doublet one-proton signals at 4.12 and 4.78 ppm with ^3J = 9.9 and 9.2 Hz correspond to lactone protons, the lines of the second signal being considerably broadened, as in the case of anabsin. Broadened one-proton doublets at 2.77 and 3.37 ppm with $^3J = {}^3J$ = 10 Hz and a singlet at 2.50 ppm are due to methine protons.

A comparison of the PMR spectra of compounds (I) and (V) has shown that on the whole they are similar. The main difference is the considerable change in the positions of the signals of the protons of the two tertiary methyl groups and the absence of the signal of a hemihydroxylic proton from the spectrum of (V).

From the results of a study of the ^{13}C NMR spectra of anabsin and anabsinthin taken under conditions of complete and incomplete decoupling from protons, the types of carbon atoms present in these compounds have been identified in relation to the nature of their hybridization and degree of substitution:

Types of carbon atoms	Numbers of carbon atoms in		
	anabsin (I)	anabsinthin (V)	absinthin (VI)
$-CH_3$	6	6	6
$-CH_2-$	4	5	4
$-\overset{\vert}{C}H-$	12	11	11
$-\overset{\vert}{\underset{\vert}{C}}-$	4	4	3
$-CH=$	—	—	1
$-\overset{\vert}{C}=$	2	2	3
$-\overset{\vert}{C}=O$	2	2	2

The carbon composition of anabsin and anabsinthin differ only by the ratio of the numbers of $-CH_2-$ and $-CH=$ groups, which is due to the presence of a secondary hydroxy group in the molecule of (I).

The spectrum of (VI) has the three-proton signals of six methyl groups: doublets at 1.00 and 1.12 ppm with ^3J = 6.7 Hz [2 (CH—CH_3)], singlets at 1.14 and 1.39 ppm [2 (HO—C—CH_3)], and singlets at 1.67 and 2.04 ppm (C=C—CH_3, HC=C—CH_3). The signal of the olefinic proton forms a broadened singlet at 5.48 ppm. The chemical shifts of the lactone protons are similar to one another and appear in the form of a multiplet with its center at 4.79 ppm (2 H). In the spectrum of compound (VI) taken in $CDCl_3$, this multiplet is observed in the form of a triplet with its center at 4.61 ppm formed by the mutual superposition of two doublets. Consequently, each lactone proton interacts with one proton in the vicinal position.

The structure of absinthin has been reconsidered, and another structure (X) has been suggested for it [5].

The features of the H and ^{13}C NMR spectra of absinthin that we have obtained contradict this structure. Thus, in the absinthin molecule we found the presence of two methyl groups on double bonds. Another contradiction is that the lactone proton at C_6 should interact with only one vicinally located lactone group.

Furthermore, the structure (X) is not in harmony with the results of a study of the ^{13}C NMR spectrum of absinthin; namely, a difference is found in the numbers of CH_2-, $-\overset{\vert}{C}H-$, and $-\overset{\vert}{\underset{\vert}{C}}-$ carbon atoms.

The contradictions would be eliminated if the double bonds in the absinthin molecule were located at the C_4-C_5 and C_{18}-C_{19} carbon atoms and the two guaiane fragments were linked with the participation of the carbon atoms C_2 and C_3, and C_{17} and C_{20}.

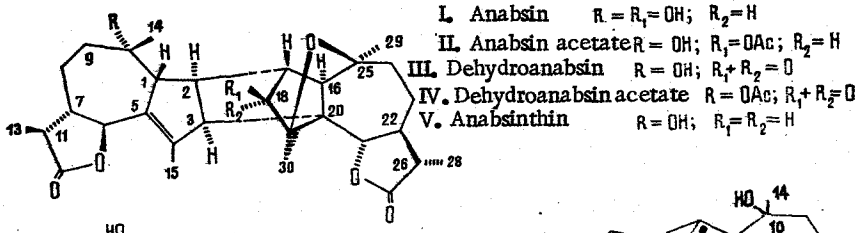
It is known that when absinthin isomerizes into anabsinthin an oxygen bridge is formed. In this process, the HC=C—CH_3 and HO—C—CH_3 fragments suffer changes. According to the ^1H and

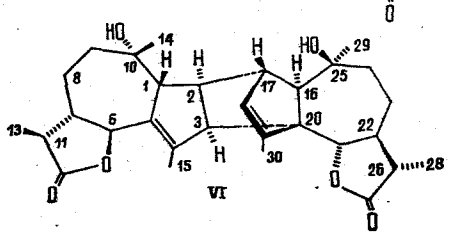
^{13}C NMR spectra of anabsinthin this bridge must be formed between C_{19} and C_{25} as is shown in the structure V.

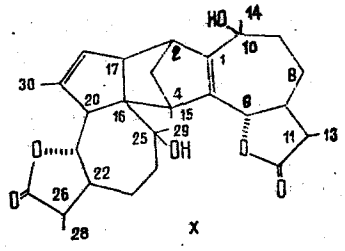
It has been observed that anabsin differs from anabsinthin by the presence of a secondary hydroxy group in the cyclopentane ring, and the only position remaining for this is at C_{18}. If the configuration of the hydrogen atoms at C_2 and C_3 is taken into account, four variants of the mutual binding of the guaiane fragments of the compounds studied are possible.

On the basis of a detailed analysis of the parameters of the signals in the ^1H and ^{13}C NMR spectra of anabasin and its derivatives, taking spatial factors into account, we suggest the binding of C_2 to C_{17} and of C_3 to C_{20} with the α orientation of the hydrogen atoms at C_2 and C_3 as shown in the structural formulas of anabsin (I), anabsinthin (V), and absinthin (VI).

The secondary hydroxy group in the anabsin molecule has the β orientation.



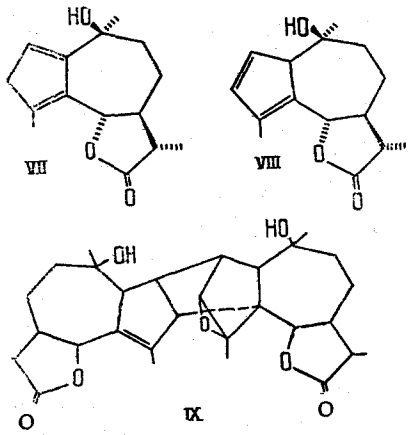




It follows from the structure of absinthin that it is a natural product of the Diels-Alder reaction formed from two molecules of the guaianolide (VIII), which, in its turn, is possibly formed from artabsin (VII) [6]. It is known that the configuration of the substituents of the diene and the dienophile does not change in the diene synthesis.

The configuration of the functional groups in the absinthin molecule and in the related molecules of anabsinthin and anabsin follow from this.

In the plant, anabsin is apparently formed from absinthin via the epoxide compound (IX).



EXPERIMENTAL

The IR spectra were taken on a UR-20 instrument (tablets with KBr), the mass spectra on a MKh-1303 instrument, and the ^1H and ^{13}C NMR spectra in deuteropyridine solution on JNM-4H-100 and Varian XL-100 spectrometers with HMDS and TMS, respectively, as 0.

Isolation of Artabsin (VII), Absinthin (VI), and Anabsin (I). The leaves and inflorescences of common wormwood (40 kg) collected in the Tashkent oblast (July, 1976) were extracted with chloroform. The concentrated extract, after the elimination of the chloroform,

was dissolved in ethanol (20 liters), and the solution was diluted with water (10 liters) and left for a day. The resulting precipitate was separated off, and the aqueous ethanolic solution was shaken successively with petroleum ether, benzene, and chloroform. Evaporation of the solvents yielded 84 g of petroleum-ether extract, 420 g of benzene extract, and 580 g of chloroform extract.

The benzene extract (420 g) was chromatographed on alumina (activity grade IV) in a ratio of 1:20. Chromatography was begun with hexane–benzene (1:1 and 1:3) and was continued with pure benzene and with benzene–acetone (99:1; 97:3; 19:1; 93:7). The fractions eluted with hexane–benzene (1:3) deposited 1.4 g of crystals with mp 133°C, which were identified as artabsin (VII). Further elution with benzene–acetone (99:1 and 97:3) gave 1.8 g of absinthin with mp 178-179°C (from ethanol).

When the fractions that had been eluted with benzene–acetone (19:1 and 93:7) were treated with methanol, 4.5 g of crystals of anabsin with mp 276°C (decomp.) deposited. On thin-layer chromatograms sprayed with a 1% solution of vanillin in sulfuric acid, anabsin was stained red-violet, changing to violet, with R_f 0.4, like absinthin (ethyl acetate system on alumina as sorbent). Anabsin, like absinthin, is very bitter to the taste.

Anabsin (I): $C_{30}H_{40}O_7$ (mol. wt. 512, mass spectrometrically). IR spectrum, cm^{-1}: 3485 (OH), 1760-1780 (C=O of γ-lactone rings), 1660 (C=C). ^1H NMR, ppm: 0.97, 1.09, 1.22, 1.31, 1.57, 1.90 — methyl groups; 4.18 and 4.74 — lactone protons; 4.07 — hemihydroxylic proton; and 2.69, 2.81, and 3.77 — methine protons.

Anabsin Acetate (II). To a solution of 0.2 g of anabsin in 4 ml of absolute pyridine was added 3 ml of freshly distilled acetic anhydride and the mixture was left at room temperature for 12 h. Then it was poured into ice water and extracted with chloroform. The concentrated extract was chromatographed in alumina (5 g). Benzene fractions yielded 0.15 g of crystals with mp 310°C (ethanol), $C_{32}H_{42}O_8$ (mol. wt. 554, mass spectrometrically).

IR spectrum, cm^{-1}: 3440 (OH), 1770 (C=O of γ-lactone rings), 1740 and 1240 ($OCOCH_3$).

^1H NMR spectrum, ppm: 1.15, 1.31, 1.40, 1.95, 1.07, and 1.09 — methyl groups; 1.83 — $OCOCH_3$; 4.16 and 4.85 — lactone protons; 5.06 — hemihydroxylic proton.

Dehydroanabsin (III). Anabsin (I) (0.5 g) was dissolved in 15 ml of pyridine and a solution of 0.5 g of chromium trioxide in 0.4 ml of water was added, after which the mixture was left at 40-50°C for 4 h. Then it was poured into ice water and extracted with chloroform. The reaction product was purified by chromatography on alumina. This gave dehydroanabsin (0.3 g), $C_{30}H_{38}O_7$, mp 295°C (decomp.), mol. wt. 510 (mass spectrometry).

IR spectrum, cm^{-1}: 3500 and 3380 (OH), 1770-1750 (C=O of γ-lactone rings and C=O of a cyclopentanone ring).

^1H NMR spectrum, ppm: 0.98, 1.09, 1.13, 1.33, 1.38, and 1.89 — methyl groups; 4.23 and 4.72 — lactone protons.

Dehydroanabsin Acetate (IV). Dehydroanabsin (III) (0.15 g) was dissolved in 8 ml of freshly distilled acetyl chloride and the mixture was left at room temperature for 4 h. Then the acetyl chloride was evaporated off in vacuum and the residue was dissolved in chloroform and chromatographed on alumina (5 g). This gave 0.05 g of dehydroanabsin acetate, $C_{32}H_{40}O_8$ (mol. wt. 552, mass spectrometry), mp 250°C (decomp.).

IR spectrum, cm^{-1}: 1715 and 1780 (C=O of γ-lactone rings), 1750 (C=O in a cyclopentane ring), 1735 and 1250 ($OCOCH_3$).

^1H NMR spectrum, ppm: 0.94, 1.07, 1.08, 1.29, 1.55, and 1.91 — methyl groups; 1.75 — $OCOCH_3$; 4.23 and 4.50 — lactone protons.

Anabsinthin (V). Absinthin (VI) (1 g) was dissolved in ether containing traces of hydrochloric acid and the solution was left at room temperature for 12 h. The crystals of anabsinthin that deposited (0.8 g) were recrystallized from methanol, $C_{30}H_{40}O_6$, mp 260°C (mol. wt. 496, mass spectrometry).

IR spectrum, cm^{-1}: 3600 and 3380 (OH), 1770-1760 (C=O of γ-lactone rings), 1660 (C=C).

^1H NMR spectrum, ppm: signals at 1.11, 1.36, and 1.93 — methyl groups; 4.12 and 4.78 — lactone protons; 2.50, 2.77, and 3.37 — methine protons.

SUMMARY

1. The new diguaianolide anabsin has been isolated from *Artemisia absinthium*, and its structure has been established on the basis of chemical transformations and an analysis of ^1H and ^{13}C NMR spectra.

2. The structures of absinthin and of anabsinthin have been reconsidered.

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SYNTHESIS AND STUDY OF HYDRAZONES OF 17α-HYDROXYPROGESTERONE AND 17α-HYDROXYPREGNA-4,6-DIENE-3,20-DIONE AND THEIR 17α-ACYLATED DERIVATIVES

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It has been shown that under mild conditions the reactions of 17α-hydroxy 3,20-diones of the pregnane series with acid hydrazides lead to the formation of 3-monohydrazones, while under severe conditions 3,20-dihydrazones are formed regardless of whether a C_4-C_5 ethylenic bond or a chain of conjugation of C_4-C_5 and C_6-C_7 ethylenic bonds is present in the steroid molecule. The reaction of 17α-acetoxy 3,20-diketones of the pregnane series with acid hydrazides takes place only with the formation of 3-monohydrazones. An investigation of the gestagenic action of some of the compounds synthesized has shown that the presence of a 3-keto group in the steroids of the pregnane series is not necessary for the retention of this effect. The replacement of the keto group at carbon atom 3 by an azomethine group does not abolish the gestagenic action.

According to the literature [1], a necessary condition for gestagenic action is the presence in the pregnane molecule of a 3-keto group and a C_4-C_5 ethylenic bond conjugated with it. Until now, it has been considered [1] that various changes connected with the replacement or reduction of the carbonyl group at C_3 either deprive the compound of gestagenic activity or greatly weaken it. However, it has been shown for a number of androstane compounds [2-6] that the replacement of an oxygen by a nitrogen atom at C_3 in androstane hydroxy ketones leads to an enhancement of biological activity.

A study of the reaction of the keto groups in an androstane 3,17-diketone with acid hydrazides [7] has shown that if the steroids include saturated rings A and B or a C_4-C_5 ethylenic bond conjugated with the 3-keto group, the reaction takes place at both carbonyls with the formation of dihydrazones. In this case, monohydrazones cannot be obtained by any changes whatever in the ratio of the initial components [7]. The presence in ring A of the

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