STRUCTURE OF HANPHYLLIN

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In a previous paper we gave the results of investigations to establish the structure of hanphyllin (I) – a new sesquiterpene lactone isolated from Handelia trichophylla (Schrenk) Heimerl [1, 2]. The nature of the oxygen-containing substituents of (I) was shown by intensive absorption bands in its IR spectrum (Fig. 1) at 3488 cm^{-1} (OH) and 1748 cm^{-1} (carbonyl of a γ-lactone ring in conjugation with an exomethylene group). Hanphyllin is acetylated by acetic anhydride with the formation of a rapidly polymerizing liquid acetate (II), which shows the secondary nature of the hydroxy group of the lactone.

In the PMR spectrum of (I), the hydroxy proton is shown by a signal the chemical shift of which depends very greatly on the solvents used. With the use of $CDCl_3$ (Fig. 2a), this group corresponds to a broadened one-proton signal at 2.87 ppm. On passing to a mixture of $CDCl_3$ and C_5D_5N (2 : 1), this signal contracts and appears in the form of a doublet with J = 3 Hz at 6.60 ppm. Simultaneously there is a change in the fine structure of a one-proton quartet with $^3J = 9.9$ and 6.3 Hz at 4.21 ppm (see Fig. 2a, a') corresponding to a hemihydroxylic proton.

In the PMR spectrum of (I) (see Fig. 2a), the signals of the protons of the exomethylene group appear in the form of doublets at 5.50 and 6.21 ppm. The assignment of the three-proton singlets at 1.39 and 1.66 ppm of two methyl groups and also of a group of signals in the 4.40-5.00 ppm region corresponding, according to the integral curve, to three protons was made by the method of multifrequency resonance. On saturating the resonance transitions of one of the methyl groups (1.39 ppm, CH_3-14) the lines of the signal at 4.87 ppm (H-1) contracted considerably (see Fig. 2b, c) and its quadruplet structure with $^3J = 4.7$ and 11.9 Hz appeared clearly. On simultaneous irradiation with the resonance frequencies of the protons of both methyl groups (1.39 ppm, CH_3-14, and 1.66 ppm, CH_3-15), in addition to the H-1 signal, the signal at 4.70 ppm (H-5) underwent a similar change (Fig. 2b-d), being converted into a doublet with $^3J = 9.6$ Hz.

On the basis of these facts, the presence of two $H_3C-C=CH-$structural elements in (I) was established. One of the olefinic protons (H-1) interacts with a methylene group in the vicinal position and the other (H-5) only with the lactone proton (H-6), giving a quartet at 4.55 ppm with $^3J = 7.8$ and 9.6 Hz, as is shown by the nature of the distribution of the intensities of the lines of the signals arising, with close values of the chemical shifts of the interacting H-5 and H-6 protons and the equality $^3J_{5,6} = {}^3J_{6,5} = 9.6$ Hz. The assignment of the signal under consideration to the H-6 lactone proton is based on the fact that on saturation of the resonance transi-

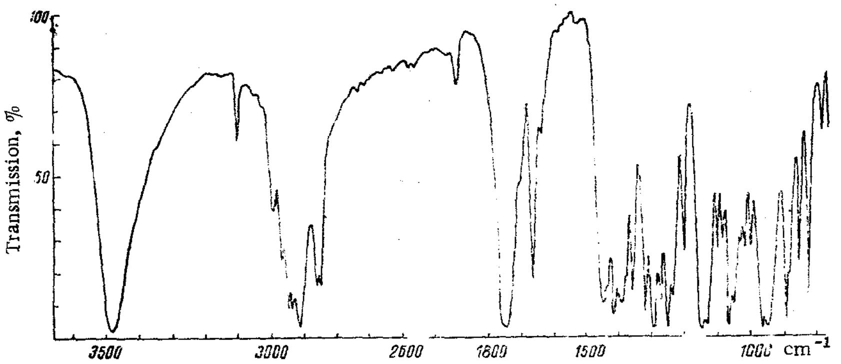

Fig. 1. IR spectrum of hanphyllin.

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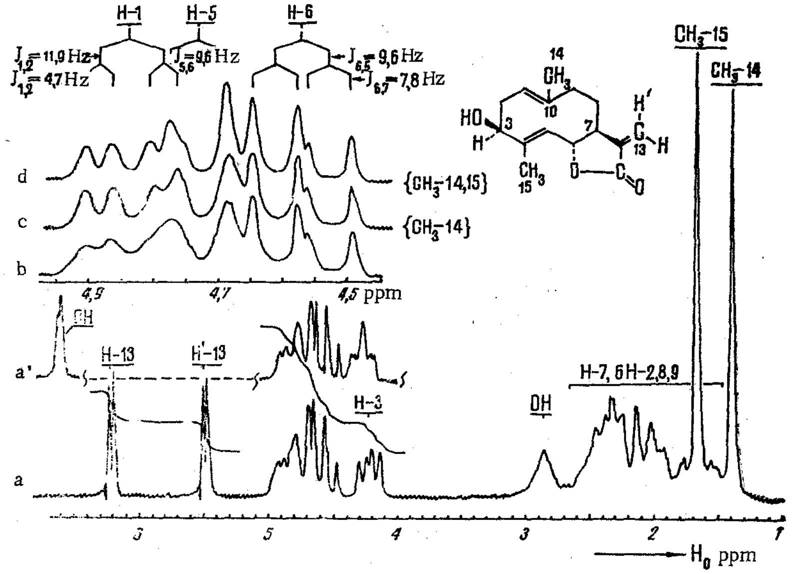


Fig. 2. PMR spectra of hanphyllin (I): a) in $CDCl_3$; a') in $CDCl_3+C_5D_5N$ (2 : 1); section of the spectrum of (I) in $CDCl_3$ in the following regimes: b) monoresonance; c) double resonance; d) triple resonance. The groups of protons irradiated are shown in parentheses.

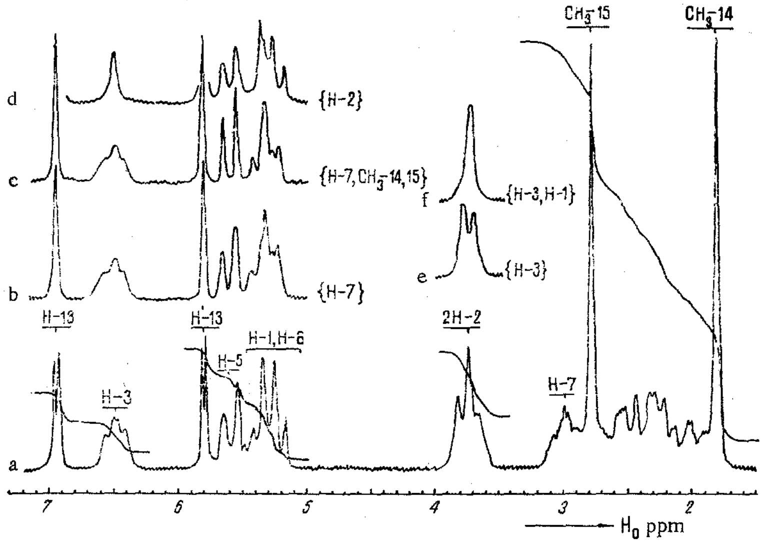
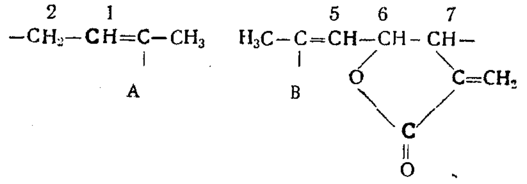


Fig. 3. PMR spectra of hanphyllin (34.8 mg, $1.4 \cdot 10^{-4}$ M, $CDCl_3$) +20.1 mg [$0.19 \cdot 10^{-4}$ M of $Eu(FOD)_3$]: a) monofrequency, b-f) multifrequency resonance.

tions of the H-7 nuclei (2.50 ppm) there is a simultaneous conversion into singlets of the doublets from H-13 and H'-13 and of the quartet at 4.55 ppm into a doublet with $^3J = 9.6$ Hz.

Thus, it may be concluded that the hanphyllin molecule contains the structural fragments A and B.



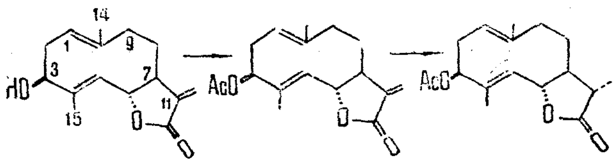
On considering the composition of (I), its structural fragments A and B, and the position in the PMR spectrum of the signal of one of the methyl groups (CH_3-14) in a stronger field (1.39 ppm), which is characteristic for lactones with the cyclodecadiene skeleton where, as the result of the transannular influence of the methyl group at C_{10} the double bond between C-4 and C-5 is screened, it may be concluded that hanphyllin has the germacrane type of skeleton.

To simplify the PMR spectrum of (I) in $CDCl_3$ ($1.4 \cdot 10^{-4}$ M) and to assign a series of signals, we used the method involving a paramagnetic shift reagent (PSR) – $Eu(FOD)_3$ (from 0 to $0.34 \cdot 10^{-4}$ M). The optimum spectrum (Fig. 3a, in which only the broadened singlet at 10.6 ppm due to the hydroxyl is not shown) was obtained on the addition of 20.1 mg ($0.19 \cdot 10^{-4}$ M) of PSR. The multiplet at 3.0 ppm was assigned to the signal of the H-7 proton, since on its selective saturation there is a broadening of the H-13 and H'-13 signals of the exocyclic methylene and of the H-6 lactone proton (see Fig. 3a, b). Additional irradiation at the resonance frequency of the H-7 protons in the vinylmethyl groups (see Fig. 3a-c) also led to a contraction of the lines of the signals of the H-5 and H-1 protons (the latter is masked by the H-6 signal).

The gradual addition of the PSR led to the isolation from the group of the multiplets present in the methylene region (see Fig. 3a) and to a downfield shift to 3.74 ppm of a two-proton signal in the form of a distorted triplet. In the double resonance spectra on irradiation with the frequency of the H-3 proton, this signal was converted into a doublet, and on simultaneous saturation of the resonance transitions of the H-3 and H-1 protons it was converted into a singlet (Fig. 3e, f). Irradiation with a frequency corresponding to the given two-proton signal led to the transformation into singlets of the signals of the H-1 and H-3 protons (Fig. 3d). Consequently, the signal under consideration at 3.74 ppm belongs to the methylene group at the C-2 atom, which interacts with olefinic (H-1) and hemihydroxylic (H-3) protons present in the vicinal position to it.

Then the hydroxy group in (I) is present at the C-3 atom and, according to the values $^3J = 9.9$ and $^3J = 6.3$ Hz has the β orientation.

The nature of the splitting of the signal of the H-6 lactone proton (quartet with $J_{6,5} = 9.6$ and $J_{6,7} = 7.8$ Hz) shows the trans linkage in (I) of the lactone ring with the main carbon skeleton at the C-6 and C-7 atoms.



I II III

Because of conjugation with a strong electrophilic function – the lactone carbonyl – the exocyclic double bond is extremely reactive. In actual fact, the reduction of hanphyllin acetate with sodium tetrahydroborate led to the formation of dihydrohanphyllin acetate (III) containing, according to its PMR spectrum, one methyl group more than the initial compound (II). The physical constants and IR, PMR, and mass spectra of (III) agree well with the literature characteristics of dehydronovanin, a derivative of novanin [3], which shows their identity. In novanin, the acetyl group at C-3 has the β orientation.

Of the compounds that have been described, tamaulipin B is closest to hanphyllin, but in the former the OH group at C-3 is α-oriented [4]. The transition from hanphyllin to the known dihydronovanin that has been performed confirms the structure and configuration of (I), which is 3-hydroxy-3α,6β,7α-H-germacra-1(10),4,11(13)-trien-6,12-olide.

Hanphyllin was later detected in the plant Artemisia aschurbajevii C. Winkl., as well [5].

EXPERIMENTAL

The IR spectra were taken on a UR-20 spectrophotometer (tablets with KBr), the PMR spectra on a JNM-4H-100 instrument (0 – HMDS, δ scale), and the mass spectra on an MKh-1303 spectrometer. TLC was performed on "Silufol R" plates in the chloroform–methanol (8.5 : 1.5) system with a 0.5% solution of vanillin in concentrated sulfuric acid as the chromogenic agent. The results of the elementary analysis of the substances corresponded to the calculated figures.

Hanphyllin (I). The fraction of the chloroform extract of the leaves of Handelia trichophylla (Schrenk), Heimerl., collected in May in the Tashkent oblast, that were soluble in 60% ethanol (230 g) was chromatographe on a column of KSK silica gel (1 : 5). After recrystallization from acetone, fractions 12-30 [eluent: benzene–hexane (2 : 1), 0.75 liter each] yielded 0.5 g of (I) with the composition $C_{15}H_{20}O_3$, mp 191°C (surface of a metal block), $[\alpha]_D^{21}$ +155.2° (c 1.03; methanol). IR spectrum, cm^{-1}: 3488 (OH), 1748 (C=O of a lactone), 1665, 1640 (double bonds). Mass spectrum: ions with m/e 248 (M^+), 230 ($M - H_2O$), 220 (M − CO), 202 ($M-H_2O-CO$).

Hanphyllin Acetate (II). A solution of 0.1 g of (I) in a mixture of 1 ml of acetic anhydride and 2 ml of pyridine was kept at 17-18°C for 5 h. The course of the reaction was monitored by thin-layer chromatography. After the usual treatment of the reaction products, (II) was obtained in the form of a colorless oil with Rf 0.84.

Dihydrohanphyllin Acetate (III). Over 15 min, 0.1 g of sodium tetrahydroborate was added in portions to a solution of 0.1 g of (II) in 15 ml of methanol. The reaction mixture was acidified with 10% acetic acid, diluted with water, and extracted with chloroform. After elimination of the solvent in vacuum, a colorless oil was obtained which crystallized on the addition of a mixture of ethyl acetate and ether. Yield of (III) 0.075 g. The crystals were dissolved in benzene and chromatographed on neutral alumina. The purified (III) had mp 136-137°C (ethyl acetate–ether) and the composition $C_{17}H_{24}O_4$. IR spectrum, cm^{-1}: 1765 (C=O of a lactone), 1735, 1250 (ester carbonyl), 1675 (double bonds).

PMR spectrum ($CDCl_3$): singlets (3 H each) at 1.40, 1.63, and 2.05 ppm; doublet (3 H) with J=7.0 Hz and 1.20 ppm, quartet (1 H) with ^3J=10 and 7 Hz and 5.1 ppm; quartet (1 H) with ^3J=10 and 7 Hz at 5.1 ppm; quartet (1 H) with ^3J=10 and 7 Hz at 4.52 ppm; broadened doublet (1 H) with J=10 Hz and 4.76 ppm; multiplet (1 H) at 4.85 ppm.

Mass spectrum: peaks with m/e 292 (M^+), 250 (M − 42), 232 (M − 60), 217 (M − 60 − 15).

SUMMARY

1. A new germacranolide, which has been called hanphyllin, has been isolated from the epigeal part of Handelia trichophylla (Schrenk.) Heimerl.

2. On the basis of an analysis of the PMR spectra using the method of multifrequency resonance and a paramagnetic shift reagent – $Eu(FOD)_3$ – the structure of hanphyllin has been established as 3-hydroxy-3α,6β,7α-H-germacr-1(10),4(5),11(13)-trien-6,12-olide.

3. Passage from hanphyllin to the known dihydronovanin has been effected.

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