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VAPOR-PHASE HYDROGENATION IN THE GLC ANALYSIS OF SESQUITERPENE LACTONES OF THE EUDESMANE SERIES

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The GLC analysis has been carried out of the products of the vapor-phase hydrogenation of the unsaturated lactoens of Inula helenium L. Tetrahydroalantolactone has been detected for the first time in elecampane rhizones and roots. During the vapor-phase hydrogenation of the compounds being analyzed in the presence of a Ni catalyst, a migration of the exocyclic double bond of isoalantolactone and of dihydroisoalantolactone was observed.

At the present time, the medical industry is producing the antiulcer drug Alanton, which consists of the total sesquiterpene lactones of the rhizomes and roots of elecampane, Inula helenium [1].

The GLC method has been proposed for the quantitative analysis of the active principles present in medicinal plant raw material and for the stagewise control of the Alanton production process [2, 3]. Six substances have been detected on a chromatogram of an elecampane extract [2].

In the GLC of authentic samples of sesquiterpene lactones isolated from elecampane rhizomes and roots [4] it was established that four of the peaks belonged to dihydroalantolactone (DA), alantolactone (A), dihydroisoalantolactone (DIN), and isoalantolactone (IA), their relative retention times (r) being 1.19, 1.34, 1.83, and 1.96, respectively (the standard substance being anthracene.

In the present paper we consider the identification of one more substance, with r = 1.62 detected on the chromatogram of elecampane rhizomes and roots.

In view of the fact that the elecampane sesquiterpenes have different degrees of saturation of the carbon-carbon bond in the eudesmane nucleus, we assumed the presence of the completely hydrogenated substance, i.e., natural tetrahydroalantolactone (TA). It has been known previously only as a product of the reduction of sesquiterpene compounds of the eudesmane series [5, 6].

With the aim of elucidating the possibility of the hydrogenation of these compounds during GLC, in place of helium we used hydrogen as the carrier gas. On comparing chromatograms of the elecampane extract obtained under identical conditions but with helium as the carrier gas in one case and hydrogen in the other, we detected a change in the ratio of the areas of the peaks of the substances under investigation. Table 1 shows the amounts of each compound in the total sequiterpene lactones calculated by the method of normalization with respect to the chromatogram obtaine don the use of helium as the carrier gas (Fig. 1a) and with respect to the chromatogram obtained on the use of hydrogen as the carrier gas (Fig. 1b).

It can be seen from Table 1 that on the use of hydrogen as the carrier gas the percentage of A present fell 6-fold while the percentages of DA and DIA in the mixture of sesquiterpene

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TABLE 1. Influence of the Conditions of Chromatography on the Ratio of the Components in the Total Sesquiterpene Lactones from Elecampane

Substance	Amounts of the components in the total lactones, %	
	carrier gas helium	carrier gas hydrogen
Dihydroalantolactone	2,03	35,10
Alantolactone	30,78	5,14
Substance with r = 1.62	0,39	20,20
Dihydroisoalantolactone	3,38	39,59
Isoalantolactone	63,42	0

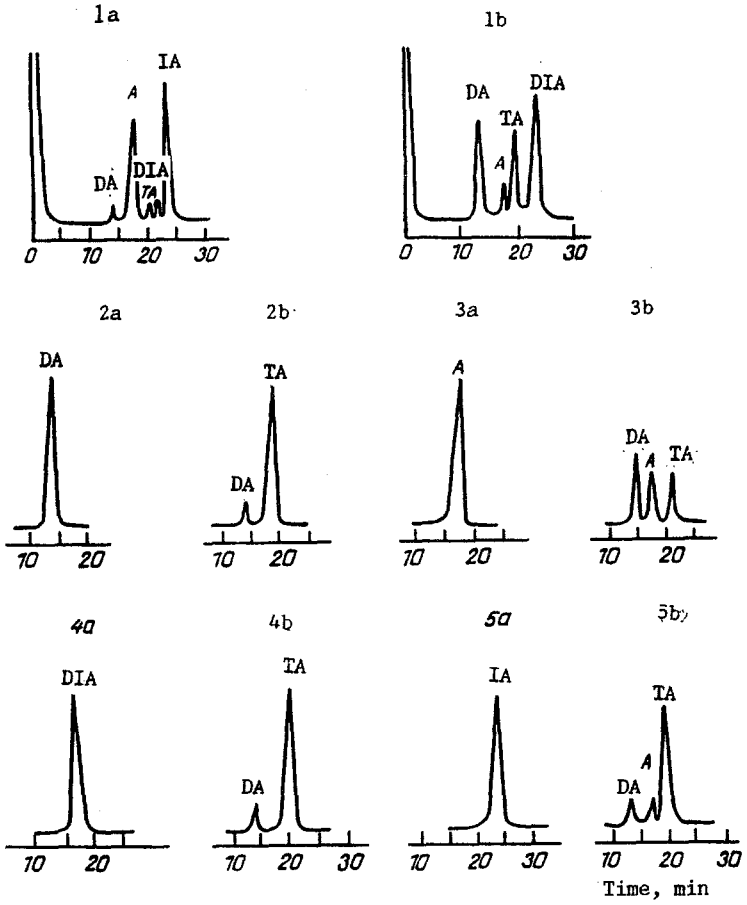
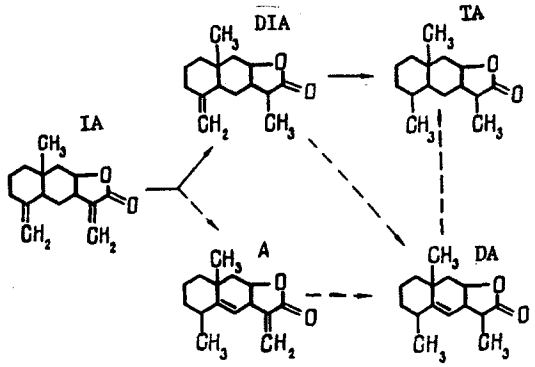


Fig. 1. Chromatograms: 1a, 2a, 3a, 4a, 5a) Extract of elecampane from the rhizomes and roots, dihydroalantolactone, alantolactone, dihydroalantolactone, and isoalantolactone, respectively; 1b, 2b, 3b, 4b, 5b) products of the vapor-phase hydrogenation of an extract of elecampane rhizomes and roots, dihydroalantolactone, alantolactone, dihydroisoalantolactone, and isoalantolactone, respectively.

lactones increased 17- and 12-fold, respectively, while IA disappeared. At the same time, the amount of the substance under investigation with r = 1.62 increased 50-fold.

The change in the quantitative ratio of the components can be explained by the assumption that the double bonds of the substances undergoing analysis were reduced. The catalyst

here was the surface of the sample-introduction block of the chromatograph, and, in all probability, reduction took place by the following scheme:



Scheme of the chemical transformations in vapor-phase hydrogenation.

To confirm this hypothesis, we used the method of vapor-phase hydrogenation with simultaneous GLC analysis. Then, from A, DA, DIA, and IA we obtained a substance having the same retention times on chromatograms recorded during the vapor-phase hydrogenation of these substances and equal to 1.62 (Fig. 2-4b). This result is evidence in favor of the assumption that the peak in a chromatogram of elecampane extracts between A and DIA belongs to TA.

A sample of TA was obtained by the catalytic hydrogenation of DIA. The hydrogenation product had the empirical formula $C_{15}H_{24}O_2$, mp 143-145°c, which corresponds to that described previously [7]. Its IR spectrum contained the absorption band at 1760 cm^{-1} that is characteristic for γ-lactones, while the absorption band in the 1647 cm^{-1} region that is characteristic for the methylene group at C_4 of dihydroisoalanolactone was absent. In the NMR spectrum of the reduced compound the methyl group attached to the lactone ring gave a signal at 1.15 ppm, while signals at 0.9 ppm corresponded to the methyl groups at C_{10} and C_4.

The GLC of the compound obtained by the hydrogenation of DIA, confirmed the assumption that the substance tested on the chromatogram of an extract of elecampane rhizomes and roots the relative retention time of which, r, was 1.62 related to TA. The quantitative determination by GLC of the TA in different samples of elecampane rhizomes and roots [2] showed that its amount ranged from 0.1 to 0.8% of the weight of the raw material.

In the performance of the vapor-phase hydrogenation of DIA we detected the formation of TA and DA. Under the same conditions IA forms not only TA but also DA and A, the subsequent reduction of which leads to TA (see Scheme). The formation of A and DA is connected with the migration of the double bond from the 4(15) in IA to the 5(6) position in A and agrees with information in the literature [5].

EXPERIMENTAL

Melting points were measured on a Kofler block. IR spectra were taken on a Shimadzu IR-27G spectrophotometer in potassium bromide tablets, and NMR spectra on a BS-497 spectrometer (Czechoslovakia) at 100 MHz, the chemical shifts being given in parts per million from HMDS as 0, and those of multiplets being measured from the center of gravity of the integral.

The GLC of an acetone extract of elecampane rhizomes and roots and of acetone solutions of A, DA, DIA, and IA was conducted on a Chrom-42 chromatograph under the following conditions: stainless-steel columns 3 mm × 2.5 m filled with Chromaton NAW-DMCS (0.20-0.25 mm) impregnated with 5% of PEGS: temperature of the sample-introduction block 200°c; temperature of the column thermostat 185°C; rate of flow of the carrier gas, helium or hydrogen, 30 ml/min.

Vapor-phase Hydrogenation. A stainless steel tube with dimensions of 0.5 × 4 cm filled with 0.5 g of comminuted nickel formate was used in a microreactor. The tube was placed in the chamber of the evaporator and in this was connected directly with the chromatographic column. The finely dispersed nickel obtained on decomposition at 200°C acted as the hydrogenation catalyst. In each case, 1 μl of a 0.2 solution of DA, A, DIA, or IA in acetone was charged into the microreactor.

Hydrogenation of Dihydroisoalantolactone with Raney Nickel. To a solution of 0.25 g of DIA in 20 ml of ether alcohol was added 0.5 ml of freshly prepared Raney nickel. The reaction was conducted at 60°C for 3 h. The solution was filtered and was diluted with water (1:1). The precipitate was separated off and was recrystallized from aqueous acetone (1:1) and dried under vacuum over P_2O_5 at 50-60°C for 4 h. This gave 0.1 g of tetrahydroalantolactone.

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STEROIDS OF THE SPIROSTAN AND FUROSTAN SERIES FROM Nolina microcarpa

III. STRUCTURE OF NOLINOFUROSIDES G AND H

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Two new glycosides which have been called nolinofurosides G(I) and H(III), have been isolated from the leaves of Nolina microcarpa. Nolinofuroside G is the sodium salt of 26-β-D-glucofuranosyloxy-(25S)-furost-5,20(22)-diene-1β, 3β-diol 1-sulfate, and nolinofuroside H is the sodium salt of 1-β-D-fucopyranosyloxy-26-β-D-glucopyranosyloxy-(25S)-furost-5,20(22)-dinen-1β-3β-ol 3-sulfate.

In preceding communications we have described proofs of the structures of five new glycosides of the furostan series and their spirostan analogs isolated from the leaves of Nolina microcarpa S. Wats. (family Dracaenacea) [1]. The present paper is devoted to a determination of the structures of the most polar components of the butanolic fraction of the total extractive substances from this plant – nolinofurosides G (I) and H (III).

Compounds (I) and (III) were colored green by vanillin phosphoric acid, and red by the Ehrlich reagent (TLC), which is characteristic of glycosides of the furostan series [2-4]. In contrast to furostan glycosides decribed previously, which were isolated in the form of mixtures of 22-OH derivatives and their 22-O-methyl ethers [1], substances (I) and (III) were isolated in the individual state. In the "fingerprint" region their IR spectra differed substantially from the IR spectra of glycosides of the furostan series]5].

The ^{13}C NMR spectrum of compound (I) lacked a signal in the 109-112 ppm region corresponding to the C-22 resonance of steroids of the furostan series [6] and contained signals at 152.47 and 103.73 ppm (Table 1). According to the APT (attached proton test), both singlets were due to the resonance of quaternary carbon atoms.

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