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SURFACE LIPIDS OF KALANHOE AS A MATERIAL FOR NANOPARTICLES PREPARATION

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The purpose of the investigation was to elaborate the methods of extraction of surface lipids from Kalanchoe Degremona plants and preparation of solid lipid nanoparticles containing a dirhenium(III) cluster compound. The procedure of growing plants and increasing the quantity of surface lipids by means of adaptation biochemistry to toxicants was used in this work. Data on the quantities of extracts, IR-spectra, and GC-MS-data of hydrocarbons and oxocompounds of surface lipids obtained were presented. An increase in the total number of surface lipids and an insignificant change in heterogeneity under the influence of monochlorobenzene exposition were shown. The absence of differences in the ratio of the intensity of the characteristic bands in the FTIR spectra allowed concluding that the toxicant did not affect the qualitative composition of the surface lipids. The nanoparticles (with a size of 145 ± 40 nm) with high encapsulation efficiency were prepared, these nanoparticles containing the dirhenium(III) cluster compound that previously showed a cytostatic action in experiments in vivo.

Keywords: surface lipids, monochlorobenzene, hydrocarbons, cluster dirhenium(III) compound, nanoparticles.

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Introduction

The complex mixture of highly hydrophobic substances situated on the surface of every land specie especially developed in not movement plants are named the surface lipids (SLs) of plants [1,2]. The functions of SLs are different but the most important ones include protection from UV irradiation, control of water status, anti-adhesive and light-reflecting properties, protection against pathogens and chemicals penetration, etc. [3]. The SLs composition differs depending on the organ and stage of plant development and the need for physiological functions accomplishment [4].

Kalanchoe Degremona (*Kalanchoe daigremontiana*, *Bryophyllum daigremontinum*) plant was chosen by us as a source of SLs due to a relatively high content of SLs and because of the phenomenon of viviparia that makes possible to work with genetically homogeneous plants. Bryophyllum species have a unique mode of vegetative reproduction, whereby young plantlets develop on the edges of leaves before being shed for propagation [5]. Furthermore, recently we have shown that some toxicants, including chlorobenzene (CB), influenced

on the SLs quantity of some plants to the side of increasing the thickness of a protective layer [1].

Nanoparticles preparation and their use in different areas of contemporary human activity are worth to mention [6]. Special attention is devoted to the solid lipid nanoparticles (SLN) due to their long and convenient storing. There is no doubt that nanoformulations are extremely valuable tools for drug delivery applications; the current challenge is how to optimize them to ensure that they are safe, effective and scalable, so that they can be manufactured at an industrial level and advance to clinical use. In this context, lipid nanoparticles have gained ground, since they are generally regarded as nontoxic, biocompatible and easy-to-produce formulations [7]. Absolute necessity of inclusion of such cytostatics as dirhenium(III) cluster compounds and cisplatin (one or both) in the lipid capsules was substantiated in our works in models of cancer genesis [8].

Thus, the aim of the present work was to elaborate the method of preparation of SLNs containing a dirhenium(III) cluster compound based on Kalanchoe Degremona SLs.

Materials and methods

Plants

Little, almost formed (with leaves, stems and roots) plants of *Kalanchoe D.* were obtained dividing them from adult plants growing in the room temperature in pots with ordinary soil. Little plants with roots were planted in previously decontaminated and roasted sand until the weight of 10–12 g. Then, they were replanted on the water solutions of CB.

All plants were divided on control (group No. 1) that grew in distilled water and exposed plants that grew on the water solutions of CB with the following concentrations: 10 mg L⁻¹ (group No. 2), 20 mg L⁻¹ (group No. 3), and 30 mg L⁻¹ (group No. 4). Each group was grown in a separate vessel and every experiment was accomplished several times. The solutions of CB for exposition were changed on freshly prepared one with the same concentrations of CB in 10 days. All plants were grown under the same light and temperature conditions. Plants of each group after 30 days were washed, dried and weighted.

All reagents used in the work were of analytical grade. The dichlorotetra- μ -isobutyrate-dirhenium(III) (Re₂(C₃H₇COO)₄Cl₂) was synthesized according to the procedure described elsewhere [9].

Extraction of SLs

SLs were extracted by hot chloroform three times, avoiding contact between chloroform and the roots, dried and weighted according to the procedure reported in ref. [1]. The results of weighting were expressed in percent with respect to the total weights of the plants as the average of five trials with standard deviation. W-criterion Wilcoxon's nonparametric tests were used to compare the parameters obtained from the control group without treatment and each group of exposition. The overall significance level was set at $p \leq 0.05$.

Fourier transform infrared spectroscopy

The samples of SLs extracted from each group was dissolved in pure chloroform, 1/4 part from each sample was taken and united for each control and each exposed plant to form one sample from each control and exposed for analysis by infrared spectroscopy. Fourier transform infrared (FTIR) spectroscopy of SLs in the h-ATR (Nicolet MAGNA-IR 550 Series II Spectrometer) was applied [1] and transmission (KBr disks) was used [1].

Gas chromatography-mass spectrometry

The SLs were derivatized with methanolic HCl (Supelco). The derivatized mixtures were analyzed by GC-MS using an Agilent 6890 gas chromatograph coupled to a 5973 mass selective detector (Agilent Technologies, Waldbronn, Germany). For GC separation, a 30 m long HP5 MS capillary column

was used (0.25 mm i.d., 0.25 μ m film thickness) with the following oven temperature program: 50°C – 1 min – 50 K min⁻¹ – 170°C – 4 K min⁻¹ – 300°C – 4 min. One microliter of each derivatized extract was injected splitless (pulsed splitless) at an injector temperature of 300°C. The ion source of the mass spectrometer operated in electron impact mode at a temperature of 230°C. Full scan mass analyses were performed to get comprehensive information on the components contained in the extracts. Data were processed automatically using a database containing 178 GC retention times and mass spectral characteristics for identification of long chain alkanes and aldehydes. In order to guarantee reproducible retention times, the GC-analysis was locked on hexadecanoic acid methylester as a time reference point [10].

Functional analysis of carbonyl compounds

2,4-dinitrophenylhydrazones of oxocompounds were obtained in the reaction with 2,4-dinitrophenylhydrazine after dissolution of the total SL in ethanol [11]. Oxocompounds were analyzed by quantitative precipitation with 2,4-dinitrophenylhydrazine. Dry hydrazones were weighed and recalculated for the content of oxocompounds. TLC of 2,4-dinitrophenylhydrazones was carried out on Silufol plates by a bottom-up method in a solvent system of diethyl ether: chloroform in a ratio of 1:10. Dry chromatograms were visualized by UV lamp, the components were washed by ether, filtered and dried. Further, the samples were analyzed on a Synapt G2-S HDMS mass spectrometer.

SLN preparation

SLN were prepared from surface lipids according to the procedure reported by Li et al. [12]. In short: dried SLs were extracted in chloroform, placed in a round-bottom flask and dried in soft conditions on a rotor evaporator to obtain a lipid film. The solution of dichlorotetra- μ -isobutyrate-dirhenium(III) Re₂(i-C₃H₇COO)₄Cl₂ in chloroform was added with the final molar ratio lipid:rhenium compound=8:1 (approximately). The solvent was dried to obtain cis-Re₂(i-C₃H₇COO)₄Cl₂-lipid film. Then, the physiological solution was added to the film and stirred for 10 min to obtain the suspension that was treated by ultrasound 10 min on Ultrasonic Perkin Elmer 3200 R to obtain SLN loaded with Re₂(i-C₃H₇COO)₄Cl₂ in suspension. Encapsulation efficiency was measured according to the procedure reported by Duong et al. [13].

Transmission electron microscopy

Surface morphology of nanoparticles was estimated by transmission electron microscopy (TEM) using JEM-1011 microscope (Japan) with

an electron kinetic energy of 100 keV.

Results and discussion

Total quantities of the surface lipids varied from 120 to 380 mg in SLs extracted from the 100 g of the fresh leaves (Table 1).

Table 1

Total quantity of SLs, g (M±m, %), (n=3; ±SD)

Control	Total quantity of SLs concentration of CB, mg L ⁻¹		
	10	20	30
0.12±0.02	0.19±0.04*	0.21±0.02*	0.38±0.02*

Note: *– P≤0.05, in comparison with control group.

The quantity of SLs is higher than from water plants extracted according to the same method [1]. The total quantity of SLs from the exposed plants has more than tripled and it was three times greater than in water plants grown in contaminated water.

This supports our previously made conclusion about activation of SLs biosynthesis by some contaminants.

The FTIR spectra of SLs from the investigated plants showed absorption bands from four groups in the wave number range from 3000 cm⁻¹ to 670 cm⁻¹ (Fig. 1).

It is necessary to underline that the total spectrum of SLs witnesses a very low quantities of carbonyl compounds in the SLs of control plants. To analyze possible differences between SLs of control and experimental plants, we discussed in detail the second derivatives of the FTIR spectra presented in Fig. 2.

Our special attention was paid to comparison between the areas of carbonyl absorption and the area of fingerprints. The bands are indicative of IR absorption of the aliphatic substances. The strong bands at 2927–2916 cm⁻¹ and 2856–2848 cm⁻¹ were assigned to valent asymmetric (ν_{as}) and symmetric

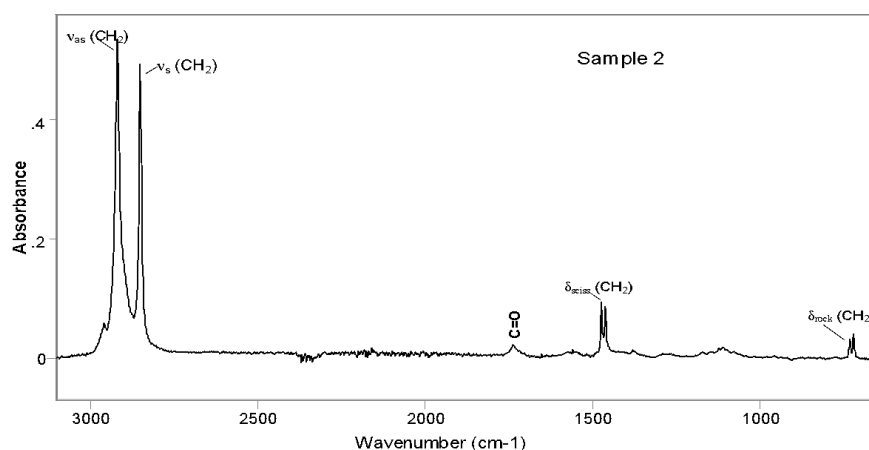


Fig. 1. Total FTIR spectrum of SL from control Kalanhoe D. plants

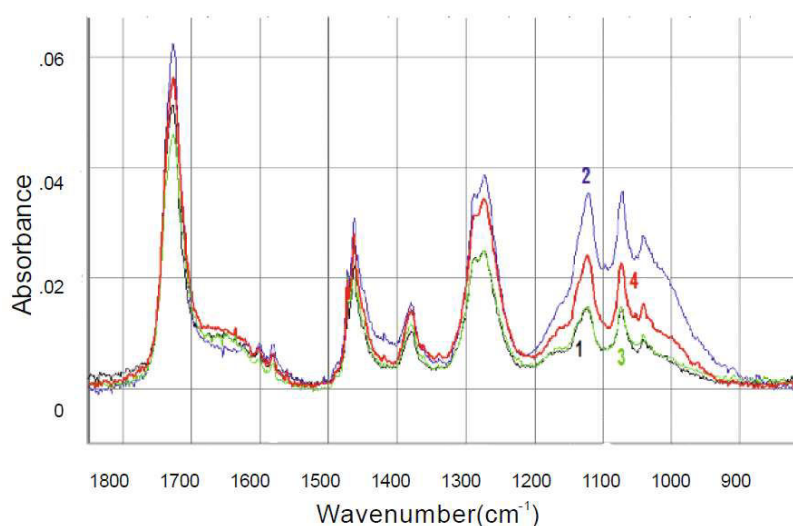


Fig. 2. FTIR-spectra of SLs: 1 (black) – 10 mg L⁻¹; 2 (blue) – 20 mg L⁻¹; 3 (green) – 30 mg L⁻¹; 4 (red) – control

(ν_s) CH_2 stretching, respectively; they are typical of plant SLs [1]. Deformational scissoring (σ_{sciss}) and rocking (σ_{rock}) CH_2 modes lead to the appearance of band doublets of intermediate intensity at 1473–1471 cm^{-1} and 730–720 cm^{-1} , respectively. Carbonyl absorption was present at 1720–1745 cm^{-1} for all samples. Earlier, the differences between exposed and control plants were found in ratio of intensity of some characteristic bands in FTIR spectra. It is clear that there were no differences in position and intensity of these bands in our experiments, which allows concluding that the toxicant influenced only on the quantity, but not the quality of the SLs of young plants.

The content of SLs from Kalanhoe D. was studied earlier [14]. The main components of the adult plants of Kalanhoe D were hydrocarbons that reached up to 90% of the total SLs [15]. Long chained fatty acids, alcohols and other components did not exceed 5% of the total quantity of SLs. According to our measurements, the main components of the obtained SLs were also hydrocarbons (Table 2).

The difference between our data and those presented earlier by van Maarseveen and Jetter [14] were as follows: (i) very little quantities (traces) of the $\text{C}_{33:0}$ alkane, that was the main component; and (ii) comparatively large quantity of even alkanes. These differences may be explained by the circumstances that the compared plants were of different age, as it is well known about changing of SL of plants during vegetation. The heterogeneity of hydrocarbons did not increase under the impact of CB exposition, as we had explored in our previous investigations, confirming the data obtained by FTIR spectra. However, the existence of the carbonyl absorption in the spectra made us necessary to undertake chemical functional analysis of carbonyl compounds in the form of hydrozones with

subsequent preparative chromatography and mass-spectral analysis. The chemical structure and mass-spectral decomposition of derivatives of two aldehydes C_{28} -aldehyde, and C_{30} -aldehydes are presented in Fig. 3.

The content of these aldehydes is rather little (1.5–2.5% with respect to the total SL weight) that explains the possibilities of their registration only by functional analysis and by slight absorption in FTIR spectra. The molecular ion peaks are characteristic signals. The peaks at $M/z=196$ and 209 are characteristic of the aromatic nitro derivative and are formed as a result of the cleavage of the phenylhydrazine group.

The next task of our work was to synthesize the nanoparticles from the dirhenium(III) compound and SLs from Kalanhoe D. According to the previously elaborated procedure, the nanoparticles were synthesized and their TEM images are shown in Fig. 4.

The average size of the nanoparticles was 145 ± 40 nm, encapsulated efficiency being 95%. Thus, relatively high encapsulation efficiency may be explained by symmetric disposition of isobutiric hydrophobic groups around the dirhenium fragments, which facilitated hydrophobic interactions with hydrocarbons.

Conclusions

We have elaborated the method of isolation of SLs from easily grown plants, which contain mainly hydrocarbons primary with odd and a little bit with even chains. We have shown that these SLs contain also traces of two fatty aldehydes; their structures were determined. The nanoparticles of 145 ± 40 nm size containing the dirhenium(III) cluster compound, which manifested a cytostatic action in previous experiments *in vivo*, were prepared with high encapsulation efficiency.

Table 2

Content (%) of the main hydrocarbons in SL of Kalanhoe D. leaves (n=3; \pm SD)

Hydrocarbons chain length	Content of the main hydrocarbons in SL of Kalanhoe D. leaves			
	control	CB 10 mg L^{-1}	CB 20 mg L^{-1}	CB 30 mg L^{-1}
C 24:0	4.38 \pm 0.21	4.61 \pm 0.22	3.42 \pm 0.16	4.90 \pm 0.23
C 25:0	4.65 \pm 0.23	5.22 \pm 0.24	4.13 \pm 0.19	5.72 \pm 0.26
C 26:0	5.64 \pm 0.26	7.14 \pm 0.35	4.22 \pm 0.20	5.89 \pm 0.27
C 27:0	5.70 \pm 0.26	7.54 \pm 0.35	4.57 \pm 0.21	11.82 \pm 0.57
C 28:0	4.46 \pm 0.21	4.81 \pm 0.24	3.34 \pm 0.16	4.37 \pm 0.20
C 29:0	12.52 \pm 0.59	11.42 \pm 0.56	11.33 \pm 0.56	18.64 \pm 0.83
C 30:0	2.75 \pm 0.10	2.05 \pm 0.10	0.90 \pm 0.05	1.68 \pm 0.05
C 31:0	47.21 \pm 2.27	47.98 \pm 2.27	56.16 \pm 2.78	38.05 \pm 1.86
C 32:0	12.70 \pm 0.59	9.23 \pm 0.35	11.93 \pm 0.56	8.93 \pm 0.35
C 33:0	traces	traces	traces	traces

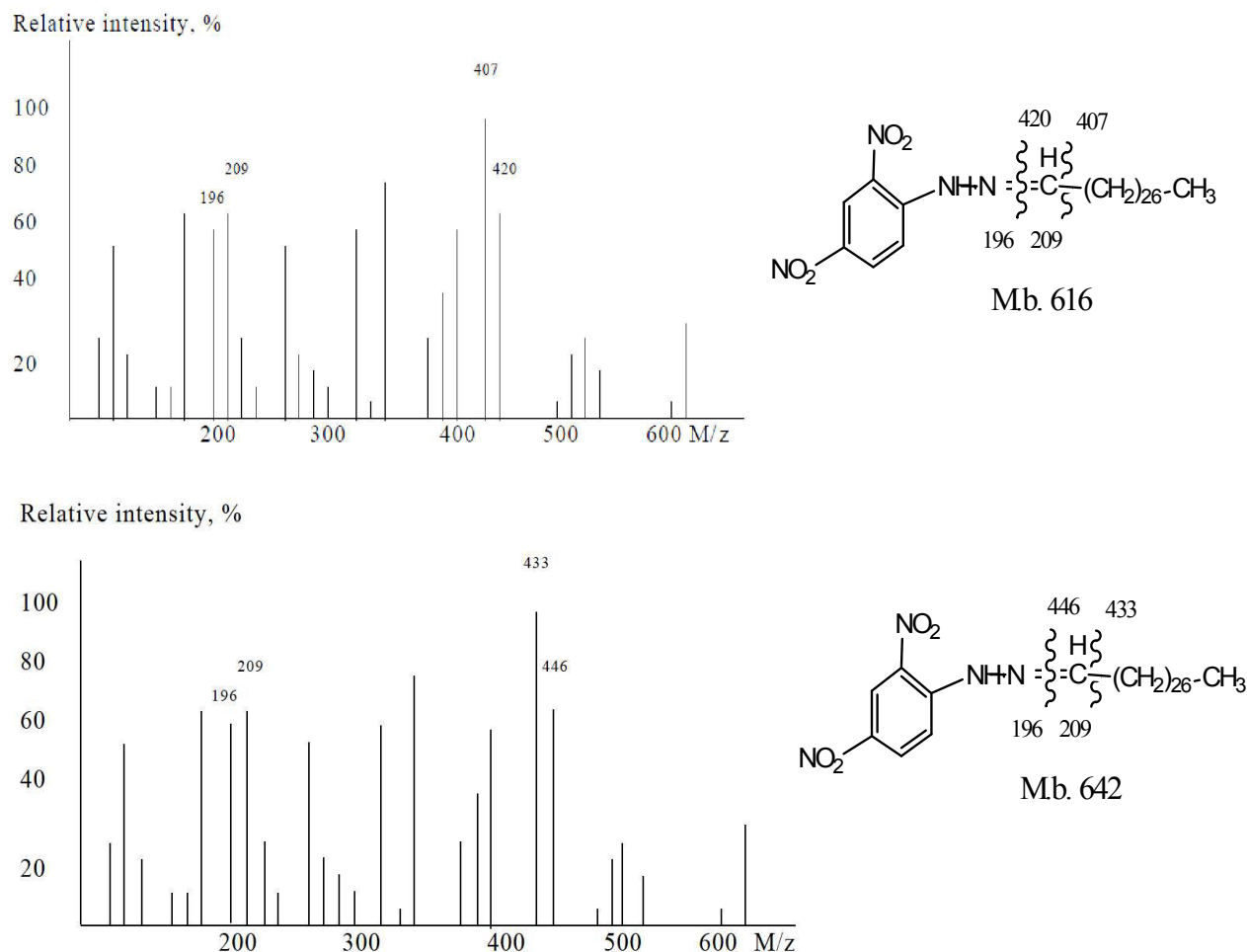


Fig. 3. The decomposition scheme and mass spectrum of 2,4-dinitrophenylhydrazone of C₂₈- and C₃₀-aldehydes

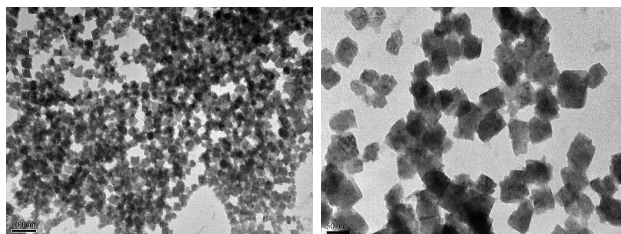


Fig. 4. TEM images of SLN obtained from Re₂(C₃H₇COO)₄Cl₂ and SLs from Kalanhoe D.

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ПОВЕРХНЕВІ ЛІПІДИ КАЛАНХОЕ ЯК МАТЕРІАЛ ДЛЯ ПІДГОТОВКИ НАНОЧАСТОЧОК

О.В. Берзеніна, Д.Є. Кітова, О.В. Штеменко, Н.І. Штеменко

Метою дослідження було розроблення методів екстракції поверхневих ліпідів з рослин каланхоє Дегремона та одержання твердих ліпідних наночастинок, що навантажені кластерною сполукою диренію(III). Була використана технологія вирощування рослин та збільшення кількості поверхневих ліпідів за допомогою біохімічної адаптації до токсикантів. Наведено дані про кількість екстрактів, ІЧ-спектри, GC-MS-дані щодо складу вуглеводнів та оксосполук поверхневих ліпідів. Показано збільшення загальної кількості поверхневих ліпідів та незначна зміна гетерогенності під впливом експозиції хлорбензолу. Відсутність відмінностей у співвідношенні інтенсивності характеристичних смуг у спектрах FTIR дозволило вказати, що токсикант не впливає на якісний склад поверхневих ліпідів. Були приготовані наночастинок (розміром 145 ± 40 нм) з високою ефективністю інкапсуляції, навантажені кластерною сполукою диренію(III), яка раніше виявила цитостатичні властивості в експериментах *in vivo*.

Ключові слова: поверхневі ліпіди, хлорбензол, вуглеводні, кластерна сполука диренію(III), наночастинок.

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