

## COMPARATIVE STUDY OF THE ANTIBACTERIAL ACTIVITY OF OINTMENT BASED ON DIPEROXYAZELAIC AND UGRESOL 10% AND ACNE STOP 20% PREPARATIONS


O. Prysiashniuk, Student

M. Blazheyskiy, Doctor of Chemistry, Full Professor  
National University of Pharmacy, Ukraine

The diperoxyazelaic acid was synthesised. Ointment on the polyethylenoxide basis containing 1% diperoxyazelaic acid was obtained. The antimicrobial activity of the ointment and branded drugs Acne Stop and Ugresol have been explored in the comparative perspective. The shelf life of creams was clarified. The conclusion was made about the prospects for further in-depth research on the issue of its application as the extemporal dosage form.

**Keywords:** acne, benzoyl peroxide, azelaic acid, diperoxyazelaic acid, antibacterial preparations.

Conference participants,  
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### Introduction

It is known that inflammations of the skin are accompanied by bacterial contamination and therefore require the use of prolonged antimicrobial action. This effect can be achieved by using external antimicrobial agents in the form of ointments, creams and gels. [1] In present among the large number of drugs proposed for treatment of acne the dosage form of surface action based on azelaic acid and benzoyl peroxide is stipulated. Azelaic acid ( $\text{HOOC}(\text{CH}_2)_7\text{COOH}$ ) – the active pharmaceutical ingredient (API) of a famous cream called Skinoren® 20% (Schering) is the antibiotic drug, which is produced by the yeast *Pityrosporum acne*. It inhibits the growth of propionic bacteria and the formation of fatty acids contributing to the occurrence of acne. In turn the API of a drug called Ugresol 10% (Pharmascience Inc., Canada), benzoyl peroxide ( $\text{C}_6\text{H}_5\text{C}(\text{O})\text{OO}(\text{O})\text{C}_6\text{H}_5$ ) effectively regulates the processes of keratinization in the sebaceous follicles, improves oxygenation of tissues, reduces the level of free fatty acids in the lipid tissues, and has antimicrobial effect, especially with regard to *Propionibacterium acne* and *Staphylococcus epidermidis* [2-10].

The research clarifies the applicability of diperoxyazelaic acid as an active pharmaceutical ingredient in preparations of surface action regarding acne, which, unlike benzoyl peroxide, is not capable of radical decomposition, and therefore will not have the ability to enhance carcinogenesis by UV radiation.

The aim of the study was to conduct the comparative study of antimicrobial activity of the ointment on the basis

of our proposed new substance – diperoxyazelaic acid (hereinafter both DPAA) (Fig. 1) and two European branded drugs – AkneStop 20% (Corporation "Arterium", Kyiv) and Ugresol Lotion 10% (Brij 30, disodium EDTA, carbomer 940) (Pharmascience Inc., Canada).

Polyethylene oxide (PEO) was chosen as the basis for ointments of diperoxyazelaic acid. This is the most common excipient of all known water-soluble bases on Pharmacopoeias of the most part of the world. The advantages of PEO: satisfactory water solubility, the ability to dissolve the hydrophilic and hydrophobic drugs, well mixed with wax and glycerides to form stable pseudo-emulsion well applied to the skin, does not prevent gas exchange and does not affect the activity of glands, weak bactericidal effect due to the presence of primary osmotically active hydroxyl groups. All PEO base are neutral, non-toxic, physiologically neutral, suitable for long-term use, not damaging the skin, easy to release drugs, not being the environment for the development of microflora. Being actively mixed with other substances, these bases can be used for all soluble and most of water-insoluble drugs.

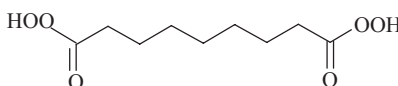
Mechanism of action of peroxyacids is the following. Compounds with active oxygen used in the composition of disinfectants and antimicrobial agents, represent hydrogen peroxide, perborate, persulfate, percarbonate etc., as well

as peracids, and hydrogen peroxide oxidation obtaining carboxylic acids. They can be seen as a replacement product or just the two hydrogen atoms in a molecule of hydrogen peroxide to acyl groups.

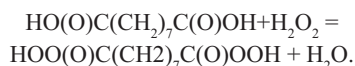
Compounds with active oxygen belong to the group of disinfectant oxidants. Under the influence of the Lane oxide group there goes oxide inactivation of lipids and proteins, essential elements of microorganisms, bacterial cytoplasmic membrane and membranes of the spore forms of bacteria. Lipid peroxidation, most pronounced on unsaturated fatty acids, leads to a decrease of hydrophobicity and increase of membrane permeability. And membrane proteins are changing through the formation of protein-lipid complexes, oxidation and denaturation of proteins containing -SH group and the possible formation of cross-links in amino ( $\text{NH}_2$ ). Inhibition of redox enzymes leads to respiratory failure of microbial cells. Study of oxygen-containing drugs on the cells of bacteria and spores of bacillus showed that all tested microorganisms lose protein up to 90%; DNA – up to 30-50%, the RNA – up to 30%, and spore forms also lose 25% of dipicolinic acid. Equally significant washout of intracellular components demonstrates the rapid violation of integrity of bacterial cells and spores [11-12].

### Materials and methods

Diperoxyazelaic acid was synthesized by a known method of Swern [13] according to the scheme (in the present sulphuric acid):



**Fig. 1. Structure of diperoxyazelaic acid**



**Tab. 1.**

**The results determine the antimicrobial activity**

Testculture	Zones of growth inhibition test cultures mm (n = 3)
<b>Ointment based DPAA 1%</b>	
<b>After 1 month of storage</b>	
<i>Staphylococcus aureus</i>	45,4±1,3
<i>Escherichia coli</i>	45,0±0,4
<i>Pseudomonas aeruginosa</i>	38,8±2,6
<i>Bacillus subtilis</i>	22,7±2,8
<i>Candida tropicalis</i>	23,7±1,4
<i>Candida albicans</i>	31,3±3,7
<i>Candida krusei</i>	21,5±1,5
<i>Candida glabrata</i>	14,8±1,7
<b>AkneStop 20%</b>	
<i>Staphylococcus aureus</i>	35,1±0,4
<i>Escherichia coli</i>	21,3±0,7
<i>Pseudomonas aeruginosa</i>	29,0±1,2
<i>Bacillus subtilis</i>	15,0±0,7
<i>Candida tropicalis</i>	22,3±0,3
<i>Candida albicans</i>	27,1±1,1
<i>Candida krusei</i>	22,4±0,6
<i>Candida glabrata</i>	9,0±0,5
<b>Ugresol 10%</b>	
<i>Staphylococcus aureus</i>	15,1±1,2
<i>Escherichia coli</i>	12,8±1,3
<i>Pseudomonas aeruginosa</i>	-
<i>Bacillus subtilis</i>	16,1±1,2
<i>Candida tropicalis</i>	17,7±0,5
<i>Candida albicans</i>	24,5±0,5
<i>Candida krusei</i>	-
<i>Candida glabrata</i>	-

Note. “-” - A zone of stunted growth of microorganisms available.

Diperoxyazelaic acid (nonanebis (peroxoic acid), T mp. + 90-90,5°C (with decomp.), the content of active oxygen species (AOC) 14,2%; pKa<sub>1</sub>=8,08, pKa<sub>2</sub>=9,19.

To confirm the stability of DPAA, one sample was kept in a self-sealing bag at room temperature (30-35 °C). The active oxygen content of the sample was determined by iodometric titration after every 10-15 days. It was found that it retains its active oxygen content over a period of 50 days (14.2-14.1%). There were no changes observed in the physical appearance, too. This confirms the stability of DPAA at room temperature.

An ointment composition: 0.8-1.2 wt. % DPAA; Poly(ethylene oxide), MW 400 (PEO 400) (Specif. 2483 007 71150986 2006), 78,0-79,4 wt.%, Poly(ethylene oxide), MW 1500 (PEO-1500) (Specif. 2483-008-71150986-2006), 20,19-19,38 wt.%; disodium EDTA (0,1-0,02 wt.%). The content of DPAA ointment is determined experimentally. Reduction of its content in the composition of the drug leads to a marked reduction of antimicrobial activity. The composition of water-soluble hydrophilic ointment base with the specified content PEO 400, PEO-1500, disodium EDTA was also chosen experimentally. Due to optimal components this framework is the most appropriate, since it promotes optimal release of active substances, chemically indifferent with respect to the drug and is convenient in terms of technology of ointments production.

The study of antimicrobial activity of the objects carried by agar diffusion was based on the ability of active substances to diffuse in the culture medium of the previously inoculated microorganisms. According to WHO recommendations we used the following museum strains for assessment of antibacterial and antifungal activity of drugs as the test cultures: gram-negative bacillus *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, Gram-positive cocci *Staphylococcus aureus* ATCC 25923 and spore-forming bacillus *Bacillus subtilis* ATCC 6633, yeast fungi *Candida albicans* ATCC 653/885, and clinical isolates: *Candida tropicalis*, *Candida krusei*,

*Candida glabrata*. In the experiment we used daily suspension of microorganisms in saline. Microbial load was 1 · 10<sup>7</sup> colony forming units in 1 ml of the nutrient medium [14,15].

Antimicrobial activity of 1% ointment DPAA was studied in 1 day 2 weeks and 1 month after preparation. The results determine the antimicrobial activity of 1% ointment DPAA. Comparison of preparations is presented in Table 1. It shows that 1% ointment DPAA manifests antimicrobial action with respect to *C. albicans* and *C. tropicalis* level comparator AkneStop 20%. While antibiotic ointment revealed the effect of DPAA against Gram-negative (*E. coli*, *Ps. Aeruginosa*) and gram-positive bacteria (*St. aureus*, *B. subtilis*) strongly exceeding the effect of other investigated drugs. As seen from the data in Table 1, ointment based of DPAA revealed high antimicrobial activity in relation to the *St. aureus*, *E. coli*,




*Ps. aeruginosa* and *C. albicans*, less pronounced – to *B. subtilis*, *C. tropicalis*, *C. krusei* and *C. glabrata*.

Consequently, studies have shown that the new DPAA ointments based on hydrophilic matrix reveal sufficiently high antimicrobial activity (which is dominated by European brands), and therefore the further in-depth research promising.

### Conclusions


The diperoxyazelaic acid was synthesised. Ointment on the polyethylenoxide basis containing 1% diperoxyazelaic acid was obtained. The antimicrobial effects of the ointment and branded drugs Acne Stop and Ugresol have been examined in comparative perspective. The shelf life of creams was clarified, and the conclusion was made about the prospects for further in-depth study of the issue of its application as an extemporal dosage form.

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## Information about authors:

1. Oleksandr Prysiazniuk - Student, National University of Pharmacy; address: Ukraine, Kharkiv city; e-mail: [prisyaga07@gmail.com](mailto:prisyaga07@gmail.com)
2. Mykola Blazheyevskiy – Doctor of Chemistry, Full Professor, National University of Pharmacy; address: Ukraine, Kharkiv city; e-mail: [blazejowski@ukr.net](mailto:blazejowski@ukr.net)



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