A PRECISE DESCRIPTION OF A POTENTIAL ANTIVIRAL AGENT AND ITS MECHANISM OF ACTION

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Abstract
A row of diseases are caused by enveloped viruses: influenza, AIDS, ebola, herpeses etc. In this paper derivatives of alpha tocopherol with an activity of membrane associated alkylating agents are proposed. They are epoxides of alpha tocopherol quinone methide, thus they can alkylate viral nucleic acids, but are easily deactivated by the cellular epoxide hydrolases.
Keywords: *tocopherol, enveloped viruses, quinone methide, epoxide*

Introduction
After the reduction of the number and severity of the diseases, caused by bacteria in the antibiotic era (what, by the way, is more and more difficult to maintain) much bigger importance got the diseases, caused by viruses. Not a small part of them is determined by infections from enveloped viruses: influenza, AIDS, herpes diseases, SARS etc. We propose derivatives of alpha tocopherol, which, theoreticaly and by a small number of initial experiments, most probably, inactivate particularly the enveloped viruses.

I. Materials and methods
The basic raw material-all-rac-alpha-tocopheryl acetate is from Merck-Germany. The activated carbon used is Norit SX Plus. TLC plates are Art. 5554 DC-Alufolien Kieselgel 60 F_{254} from Merck-Germany. The HPLC column is LichroCART 125x4 Lichrospher Si 60 (5 μm) from Merck Millipore. The silicagel for column chromatography used is Silicagel 60 (70-230 mesh ACTM) from Merck-Germany.
For the observation of the spots on TLC plate UV lamp 254 nm for thin layer chromatography (Sigma-Aldrich) is used. For the HPLC-MS analysis a device Shimadzu LCMS-8050 is used.

II. Synthesis
10 g of all-rac-d,l-alpha tocopheryl acetate (better natural enantiomeric pure alpha tocopheryl acetate) are dissolved in 100 ml absolute ethanol and a solution of 5 g potassium hydroxide in 50 ml absolute ethanol are added. The solution is stored at 25 ^{0}C approximately for 24 hours. To the solution portionwise for an hour 100 g of activated carbon with more than 800 m^2/g specific area are added. To the mixture in a course of 10 hours with swirling by portions of 10 ml totaly 700 ml of water are added. The free tocopherol (by calculations[1,2]) is sorbed so that each molecule is separated from the others. The mixture is filtered off and the wet cake is suspended in 700 ml of 0,5N potassium hydroxide. 5 g of potassium ferricyanide are added and after an hour stirring, the suspension is cooled to 0-4 ^{0}C and portionswise in a course of 10 hours with stirring at 0-4 ^{0}C totaly 80 ml of 30 % hydrogen peroxide are added. At those conditions initially alpha tocopherol quinone methide is formed [3] and, because the molecules are separated, they don't dimerize. The second stage of the reaction is epoxidation of the methylene groups near the ketogroup from the phenolic hydroxyle formed (5-th or 7-th position)[4]. The overall yield vary from 40 to 60 mol%. The suspension is filtered off and the cake is washed with water and dryed at 60 ^{0}C in vacuo. The dry powder is extracted with totaly 500 ml acetone and the extract is evaporated to an oil in vacuo.

III. Analysis
1. TLC-this is made as a two dimensional TLC by the next way: on the silicagel 60 TLC plate

15x15 cm on the left bottom angle a solution containing 200 ug of the above oil is put on. The chromatogram is developed in one direction by a mobile phase heptane:acetone=3:1. After drying the plate is stored over 25% ammonia for 24 hours. After drying again (no ammonium odor) the chromatogram is developed in a perpendicular direction by the same mobile phase, dryed and the spots are observed at 254 nm and visualised by iodine vapors. There are a few spots what are out of the diagonal, basically at Rf=0,65 in the first direction. This is the main desired product which did react with ammonium vapors[5].

2. HPLC-MS

Column: 125x4 silicagel 60 5 μm column

Mobile phase: hexane:MTBE=10:1

Flow rate: 0,5 ml/min

Detection: electrospray MS detector

The molecular mass of the desired product is 444 dalton (approx.)

IV. Purification

This is made by a column chromatography on silicagel 60;70-230 mesh (100 ml per gram of above oil). Mobile phase: hexane: MTBE = 5:1. Fractions are analyzed by HPLC-MS and the rich fractions are evaporated to an oil.

V. Mechanism of action

The product is active against enveloped viruses: influenza, HIV, herpes simplex 1,2 and zoster etc. Because of its high nonpolarity [6] it is concentrated in the lipid bilayer of the cell membranes and thus in the processes of penetration and budding of the virus particles it is able to alkylate their nucleic acids. It was found that the molecules of the product are enough small to get over the barriers of the matrix and capsid proteins [7, 8]. Quantum mechanical calculations (ChemOffice2002) show, that epoxide group reacts irreversibly with a part of nitrogen containing functional groups of the nucleic acids. The alkylated DNAs (RNAs) are blocked and can not neither replicate, nor trancript into the DNA of the host cell. In the every type of the enveloped viruses the processes of inactivation differ significantly. At the same time it is very important, that the product is not dangerous for the eucariotic cell because of the presence in the cytosolic microsomal fraction of epoxide hydrolase, what reduce the alkylation activity to zero [9]. The biological properties of the product in vivo, as toxicity, enough active dose etc. must be studied additionaly.

Results and discussion

The mixture of isomers purified from the non epoxidated admixtures after removal of the solvents is a heavy oil with not determinable melting point. For the treatment of labial and genital herpes in volunteers we made a 1 % solution of the product in vaseline. When the treatment is started in the prodromal stage a lesion doesn't appear. If the treatment is started in the first day of the lesion the time of healing is shortened to 2-3 days. Both theoretically and by experimental data the product is recommended for additional investigations.

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