ABSTRACT

DOCTOR DEGREE THESIS
SYNTHESIS, PHYSICAL – CHEMICAL CHARACTERIZATION AND PHARMACOLOGICAL TESTING OF SOME NEW CHOLESTEROL DERIVATIVES

Scientific coordinator
University Professor Dr. Stelian Radu

Doctor degree candidate,
Pharmacist Elena Dumitru

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The present work is based on the synthesis and characterization of some sterol esters with special properties. To many sterol derivatives, mainly to the cholesterol esters was noticed that they have liquid crystal (L.C.) properties.

The applications of the thermotropic liquid crystals are currently numerously ones and they cover large areas of interest: in the visible or fluorescent, in the nuclear magnetic resonance spectroscopy, in the electronic paramagnetic resonance, in neutrons dispersion, in medicine etc.

The work was structured in two parts. The first part presents a literature synthesis referring to the synthesis methods of the sterol derivatives and their potential pharmacological effects. In the experimental part of the work were synthesized and characterized thirteen cholesterol derivatives (5 cholesteryl-butyrates, 4 cholesterol carbonates and 4 cholesterol carbamates) that show a selective reflection in the solvents from which they were re-crystallized, anticipating thus a liquid crystal behavior. Through the sterol carbonates synthesis with azometin structure at C-3 sterol it was tried to obtain some sterol dimesogenes compounds that have in their structure two units: steroid carbonate and Schiff base, both units being types of liquid crystals. It was also evaluated their biological activity and their toxicity on the vegetal cell.

Many sterol derivatives, mainly the cholesterol esters present properties of liquid crystal (L.C.) To such substances, the solid – liquid phase transition is not direct, between the solid phase and the liquid phase appearing many intermediary stable phases. In such phases the substances are anisotropic as solid crystals, having nevertheless the fluidity property that is characteristic to the solids. Due to these properties they were given the name of liquid crystals, mezo-phase, mezo-morphic phases or condensed fluid phases with spontaneous an-isotropy.
The mezogenes derived from la 3β–sterol present a special interest because they are prepared from materials having vegetal or animal origin that is easily accessible, offering a wide range of applications.

The condition needed to form the cholesterol mezzo-phase is the quirality of the molecules. Because many compounds that have the capacity to form such mezzo-phases derive from the cholesterols, they were given the name cholesterol liquid crystals.

New cholesterol butyrate’s are synthesized in conformity with the methods described below. They are:

1

Cholesteryl tiophenoxi-butyrate

2

p-cholesteryl chlortiophenoxibutyrate
For their synthesis were used as raw materials the 4-tiophenoxybutir acids substituted in the position \textit{para} of the cholesterol.
The sterolic carbonates 17 and 18 with azometinic structure at C-3 sterolic were synthesized starting from 4-(4-metoxybenziliden) – aminophenol, 4-(4-metilbenziliden)-aminophenol and cholesteril chloroformiat.
Thus, through the carbonates synthesis 17 and 18 it was tried to obtain the sterolic compound that have in their structure two units: steroidic carbonate and Schiff base, both units being liquid crystal types.

Sterolic carbonates are synthesized with azo groups. The compounds 23 and 24 were synthesized starting from azo-derivates 4- (phenoxatiin – 2- azo) – phenol (25) and 4-(dibenz-p-dioxin-2-azo)-phenol (26) with cholesteryl chloroformiate in the presence of pyridine as acid acceptor.

![Chemical structures for compounds 23 and 24]
In the present work will be synthesized new cholesteryl carbamates.

Cholesterol carbamates 35 was synthesized by two methods: by the condensing of cholesteryl chloroformiate with o-iodo-aniline (39), in anhydrous benzene and in the presence of pyridine as acid acceptor, and directly throughout the reaction o-iodo-phenilazocyanate with cholesterol, in toluene.

Cholesterol carbamates 36-38 were synthesized by condensing the cholesteryl chloroformiate with amines: 2-metil – 5 – iodoaniline , 2-metil-5-nitroaniline, 2-nitro-4-methoxianilne, in the presence of pyridine as acid acceptor.
The progress of the reactions and the purity of the compounds 35-38 were controlled by TLC, by using as eluent a mixture: petrol ether: ethyl ether 5:3. The recrystallization of cholesterylcarbamates was done from a mixture benzene: alcohol.

In chapter 4 is monitored the study of the anti-microbes activity of the synthesized compounds. For the evaluation of the antimicrobial activity of the sterolic compounds were used the following test micro-organisms: *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, and *Candida albicans* ATCC 10231. The comparing inhibiting action was determined through the diffusion-metric method *Kirby-Bauer* in agar established by the National Committee for Clinical Laboratory Standards (NCCLS).

The antibacterial activity on *Escherichia coli* was shown both in the case of the carbamates 35-38 and also in case of cholesterylbutyrate 1-5 and carbonates 17-18 and 23-24, with the difference that the inhibiting action on the growth and development of the tested micro-organism is more obvious in case of the compounds 17-18, fact illustrated by the diameters of the inhibition areas that do not exceed 19 mm.

In case of the study of the anti-bacteria activity of the carbonates 23-24 it was noticed besides the big inhibiting activity of the positive-gram bacteria (*Escherichia coli* and *Staphylococcus aureus*) that these two derivatives, unlike other compounds
studied, show also a special anti fungi activity (7 mm in the inhibition diameter for compound 23, respectively 9 mm inhibition diameter for compound 24).

This fact is due to the fact that phenoxatiin and a series of its derivates are known for having a bacterial, anti-inflammatory and anti-fungi activity etc.

In chapter 5 is tested the action of the NEW CHOLESTEROL DERIVATES AGAINST THE VEGETAL CELL.

The phyto-biological testing of the new synthesized compounds evaluates certain aspects linked to the eventual cito-genity and cito-toxicity of the products obtained. The testing of the action of the chloroform solutions obtained from the synthesized compounds upon the radicular extension and on the myotic film was done by the phyto-biological method – test Triticum of professor dr. Grigore Constantinescu. Observations were done on the morphological modifications (aspect and length of epicolyte, aspect and length of radixes).

It was noticed that all solutions of the synthesized compounds inhibit with a certain percentage, bigger or smaller, the growth in length of the main root. Thus for the cholesteryl derivative 19 (4- (4- methoxibenzilidene)-aminofenoxi-cholesterylcarbonat) it is noticed a total inhibition of the main root by approx. 68% from the average of the root of the witness sample. Therefore

- All the compounds 1-5 anticipate the behavior of a liquid crystal, because they show a selective reflection in the solvents from which they were re-crystallized.
- Also in the study of the antibacterial activity done on S. aureaus, E. coli and C. albicans it was noticed a very good inhibiting action on the first two bacteria of the compounds 1-5, but no anti-fungi action of them.
- The compounds 17-18, 23-24, 35-38 inhibited the development of the bacteria S. aureaus, E. coli and compounds 23-24 had anti fungi activity. This fact can be explained through the fact that these are derivates of fenoxatiin that present a special anti fungi activity.
In the phyto-biological experiments was noticed the inhibition of the germination of species *Triticum sativa*, all compounds synthesized showing a cito-toxic potential. The test of the chromosome aberration in radicular ana-telephases (chromosome fragments, bridges, micronuclei, retarded chromosomes, disorganized ana-phases, other types of structural-chromosome anomalies) showed a moderated clastogene genetic effect (geno-toxic) that worth considering.

**SELECTIVE BIBLIOGRAPHY**

12. L. E. Vîjan, C. Topală, Rev.Chim. (Bucureşti), **2008**, 59(7), 756-758
