



Synthesis and *in vitro* Antisickling Activity of Some Non-aromatic Esters

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Authors' contributions

This work was carried out in collaboration between all authors. Author DDT designed the study, wrote the first draft of the manuscript. Author KTNN performed the statistical analysis, wrote the protocol. Authors DSTT and AMM managed the analyses of the study. Author PTM managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Organic acids and their derivatives like butyl stearate have recently shown antisickling activity. Some none aromatic esters were synthesized and tested for their antisickling activity using Emmel test. On 21 esters tested only three showed no antisickling activity. Butyl palmitate, propyl palmitate, butyl stearate, propyl stearate and methyl nonadecanoate showed interesting antisickling activity with a normalization rate higher than 70%.

Keywords: Non- aromatic esters; antisickling activity; synthesis; butyl stearate.

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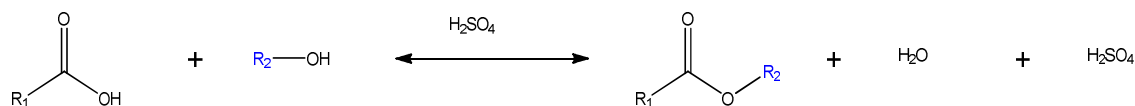
1. INTRODUCTION

Sickle cell disease (SCD) is a genetic disorder caused by an abnormality of hemoglobin. The disease is characterized by a chronic hemolytic anemia. The search for affordable and accessible medicines mainly from plants and having various modes of actions for managing SCD is a priority in Africa where the disease is endemic [1,2]. Recently our research team showed that many medicinal plants used in traditional medicine in Democratic Republic of the Congo (DRC) to manage SCD had *in vitro* antisickling activity and that this activity is mainly due to anthocyanins [3-5]. Several flavonoids like anthocyanins are natural products with a range of biological activities including free radical scavengers and antioxidant activity [6-8]. Our previous studies have also identified organic acids and butyl stearate as antisickling agents [9,10]. Some *in vitro* experiments on the antisickling activity of amino acid benzyl esters revealed that this class of compounds may have considerable utility as therapeutic agents for sickle cell disease [11].

The present study was performed with the aim of synthesizing and evaluating the antisickling activity of different non aromatic derived esters. Most of these esters can be found in pure form in the trade, but one of the aims of this study was to show among other things that they can be also simply synthesized to minimize the production

Equation of the reaction of esterification is as follow:

Esterification of fatty acid with alcohol in the presence of sulphuric acid as catalyst lead to the formation of esters as shown in the following equation:



The water formed in the reaction was separated by continuous distillation, using the Dean Stark apparatus. Progressive heating of the medium was carried out to accelerate the reaction. After esterification, the reaction medium was washed with water to eliminate residual alcohol, mineral acid (H₂SO₄) and acetic or formic acid. The ester was separated by drying with anhydrous Epsom salt to remove the water. The identification of the synthesized product was carried out using gas chromatography (GC) and gas chromatography coupled to the mass spectrometry (GC-MS). The gas chromatography was carried out using AGILENT apparatus with a chromatograph equipped with a column of 15m filled with 5% of the dimethyldiphenyl siloxane with flame ionization detector (FID). Identification of synthesized products was done using the Willey data base.

cost of the probable future antisickling drugs. For the best of our knowledge, these esters have not yet been scientifically investigated for their antisickling properties.

2. MATERIALS AND METHODS

2.1 Esters Synthesis

The method used is the esterification with sulphuric acid as catalyst, by taking into account of the physicochemical properties of the reagents and the products' reaction. For all the reactions, the fatty acids used are from technical source. In order to move the reaction balance, an initial alcohol excess in the reactional medium (for the reactions using the fatty acids) and an excess of acid for the reactions using the simple acids (acid formic and the acetic acid) with heavy alcohols were used. Water or the possibly formed azeotrope was eliminated by continuous distillation, using the Dean Stark apparatus. The progressive heating of the reactional medium was carried out to accelerate the reaction. After esterification, the reactional medium was washed with water to eliminate residual alcohol, the mineral acid (catalyst or H₂SO₄) and the simple organic acids (acid formic, acid acetic). The ball to be elutriated was used to separate esters from the remainder of the reactional medium and the water was dried out using the anhydrous Epsom salt.

2.2 Biological Experiments

2.2.1 Biological material

Blood samples used to evaluate the antisickling activity of the compounds in this study were taken from known SCD adolescent patients attending the "Centre de Médecine Mixte et d'Anémie SS" located in Kinshasa area, DRC. None of the patients had been transfused recently with Hb AA blood. All antisickling experiments were carried out with freshly collected blood. In order to confirm their SS nature, the above-mentioned blood samples were first characterized by Hemoglobin electrophoresis on cellulose acetate gel, as previously reported [5]. They were found to be SS blood and were then stored at $\pm 4^{\circ}\text{C}$ in a refrigerator. An informed consent was obtained from all the patients participating in the study. All the research procedures have received the approval of Department of Biology Ethics Committee (N. Réf.: CDB/FSC/MMJ/039/MM/2015).

2.2.2 Antisickling assay

Sickle cell blood was diluted with 150 mM phosphate buffered saline (NaH_2PO_4 30 mM, Na_2HPO_4 120 mM, NaCl 150 mM) and mixed with an equivalent volume of 2% sodium metabisulfite. A drop from the mixture was spotted on a microscope slide in the presence or absence of different esters and covered with a cover slip. Paraffin was applied to seal the edges of the cover completely to exclude air (Hypoxia). Duplicate analyses were run for each compound. The RBCs were analyzed using a computer assisted image analysis system (Motic Images 2000, version 1.3; Motic Chine Group Co LTD) and statistical data analyses were processed using Microcal Origin 7.5 package software.

3. RESULTS AND DISCUSSION

Figs. 1 and 2 show the microscopic image of sickle red blood cells alone and in presence of butyl palmitate respectively.

Mass spectrum data of the synthetic esters are in accordance with those of the literature indicating that the desired products were obtained [12].

Fig. 1 shows that the control contains in majority sickle-shaped erythrocytes, confirming the SS nature of the blood. Mixed together with butyl palmitate, the majority of erythrocytes are reversed normal-shape (Fig. 2). This indicates that butyl palmitate have antisickling effects

confirming the result already obtained with butyl stearate [4]. Antisickling activity of other none aromatic esters is given in Table 1.

Table 1. Antisickling activity of none aromatic esters

Carbon number	Esters	Antisickling activity
4	Ethyl acetate	-
10	Methyl nonanoate	+
11	Methyl decanoate	+
12	Methyl undecanoate	+
14	Methyl tridecanoate	+
15	Methyl myristate	+
16	Methyl pentadecanoate	++
17	Methyl palmitate	+
18	Methyl heptadecanoate	+
18	Ethyl palmitate	+
19	Methyl Stearate	+
19	Propyl palmitate	+++
19	Methyl Oleate	-
20	Methyl nonadecanoate	+++
20	Butyl palmitate	+++
20	Ethyl stearate	+
20	Ethyl Oleate	-
21	Methyl eicosanoate	+
21	Propyl stearate	+++
22	Methyl heneicosanoate	++
22	Butyl stearate	+++

Legend: -: negative test; +: Normalization rate < 50%; ++: Normalization rate >50%; +++: Normalization rate >70%

Structures of most active compounds are given in Fig. 3.

As it can be seen in this table, on 21 non-aromatic esters tested, only three showed no antisickling activity. The compounds which displayed interesting antisickling activity were: butyl palmitate, propyl palmitate, butyl stearate, propyl stearate and methyl nonadecanoate with a normalization rate higher than 70%. The most active compounds are those having more than 18 carbon atoms. The hydrophobicity of these long hydrocarbon chains would allow them to easily cross the membrane barrier and interact with hemoglobin S thus preventing its polymerization. Furthermore, Gorecki et al. [11] showed that aminobenzyl esters have antisickling activity and that this activity could involve both binding to deoxyhemoglobin S and modification of the erythrocyte membrane. So,

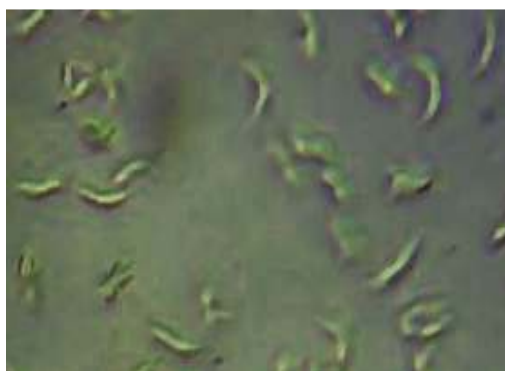


Fig. 1. Microphotography of SS blood alone (control) (x500) [NaCl 0.9%; Na₂S₂O₅ 2%,]



Fig. 2. Microphotography of SS blood incubated with Butyl palmitate (50 µg/ml) (x500) [NaCl 0.9%; Na₂S₂O₅ 2%,]

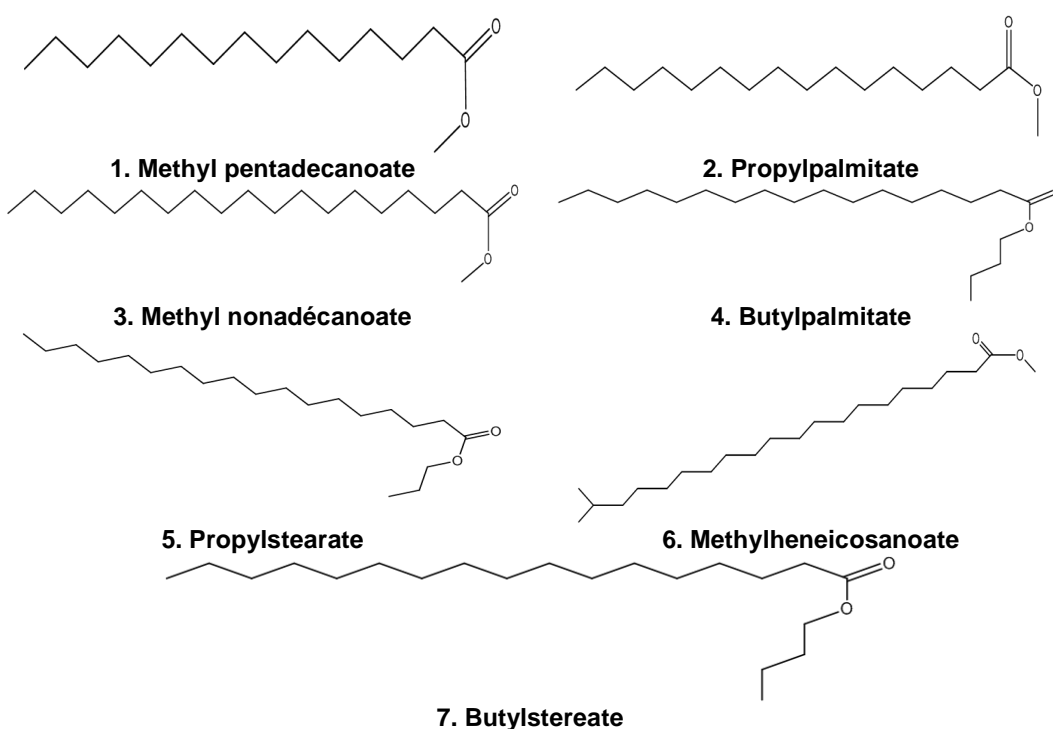


Fig. 3. structures of most active molecules: Methyl pentadecanoate (1); Propylpalmitate(2); Methyl nonadecanoate (3); Butylpalmitate(4); Propylstearate(5) Methylheneicosanoate(6) Butylstearate(7)

other tests such hemolysis, osmotic fragility, antioxidant have to be done in order to confirm this biological activity and to determine the probable mode of action of these non-aromatic esters.

4. CONCLUSION

The results outlined in this paper, demonstrate the antisickling effects of non-aromatic esters

derivatives indicating that these substances are attractive potential candidate for sickle cell disease therapy. It is therefore necessary to evaluate the interaction of bioactive esters with sickle erythrocytes membrane through osmotic fragility bioassay in order to elucidate their modes of action. The bioactivity validation of these bioactive compounds would permit their formulation as medicine the treatment of sickle cell anemia after toxicological studies.

ETHICAL APPROVAL

All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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