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Syntheses of new cyclodextrin complexes with oleanolic and ursolic acids

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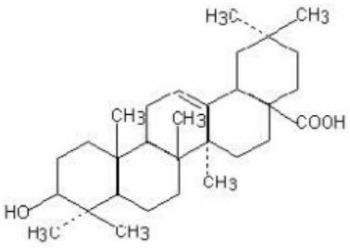
Abstract

Oleanolic acid (OA) and ursolic acid (UA) are triterpene acids with a well-known antitumor activity. The obtaining procedure (easy extractions from many plants) is an explanation for the large number of studies on their anti-inflammatory, antiviral, hepatoprotective activity, hair and photo-aged skin treatments. The low water solubility of pentacyclic triterpenes, which determine their low bioavailability, is a problem which can be solved by the complexation with cyclodextrins (CDs). In this study OA and UA inclusion complexes were prepared using two different CDs: hydroxy-propyl-beta-cyclodextrin (HPBCD) and hydroxy-propyl-gamma-cyclodextrin (HPGCD) and kneading as complexation procedure. The products were analysed by differential scanning calorimetry and X ray diffractometry and it can be concluded that the complexation with CDs is a good pathway to increase the OA and UA bioavailability.

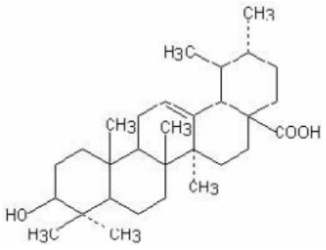
Keywords: oleanolic acid, ursolic acid, cyclodextrin, inclusion complex

1. Introduction

Triterpenes are compounds with the molecular formula $C_{30}H_{48}$, which contain six isoprene units. The pentacyclic triterpenes can be classified in three groups (lupane, oleanane and ursane) [1].



(a)



(b)

Figure 1. The chemical structure of (a) oleanolic acid (OA), (b) ursolic acid (UA)

OA (3β-hydroxy-olea-12-en-28-oic acid) and UA (3-β-hydroxy-urs-12-en-28-oic acid) (figure 1), two triterpene acids which can be purified from several botanical drugs, present a well-known antitumor activity. These compounds were already examined for their ability and possible pathway on inhibiting the tumor growth of hepG2 cells.

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The results suggested that the apoptosis induced by OA and UA might be partially mediated through PKC pathway and associated with p-15INK4b gene induction [2].

It has been shown [3] that OA and UA inhibit the Epstein-Barr virus activation induced by 12-O-tetradecanoylphorbol-13-acetate (TPA) in mouse skin. The inhibitory effects were evaluated by continuous application of the two triterpene acids before TPA-treatment and it was observed the delay in the formation of papillomas in mouse skin and the reduced rate (%) of papilloma bearing mice. Both OA and UA exhibited remarkable inhibitory activity against tumor promotion, which is comparable to the known tumor inhibitor, retinoic acid (RA) [3]. Topical cosmetic preparations containing OA or UA have been patented in Japan for the prevention of topical skin cancer [4]. An OA/UA ointment inhibited 7,12-dimethylbenz[a]anthracene (DMBA)-induced skin cancer in mice. Reportedly, 0-3% of mice developed cancer in 15 weeks and 30 weeks, respectively compared to 50-90% for the control mice [4].

OA can be extracted from the leaves and roots of *Olea europaea*, *Viscum album* L., *Aralia chinensis* L. and over 120 other plant species [5]. OA exhibits anti-inflammatory, antitumor, antiviral, hepatoprotective and anti-hyperlipidemic effects and it was used in Chinese medicine to treat liver disorders for over 20 years [6]. Conventional formulations of OA are tablets and capsules [7]; however, OA's poor aqueous solubility and low bioavailability *in vivo* make it necessary to develop new formulations for clinical applications.

UA can be extracted from many plants, including apples, basil, bilberries, cranberries, elder flower, peppermint, rosemary, lavender, oregano, thyme, hawthorn, prunes. Apple peels contain large quantities of ursolic acid and related compounds [8]. UA inhibit various types of cancer cells by inhibiting the STAT3 activation pathway [9,10] and human fibrosarcoma cells by reducing the expression of matrix metalloproteinase-9 by acting through the glucocorticoid receptor. It may also decrease proliferation of cancer cells and induce apoptosis [11]. Different treatments with UA improve the health of skin and hair.

Another action is the photo-aged skin treatment because UA prevents and improves the appearance of wrinkles and age spots by restoring the skin's elasticity and collagen bundle structures [12].

It has been found to reduce muscle atrophy and to stimulate muscle growth in mice [13,14]. Ursolic acid has potential use as a cardioprotective compound [15] and it was found to be a weak aromatase inhibitor (IC50 = 32 μm) [16]. UA can serve as a starting material for synthesis of more potent bioactive derivatives, such as anti-tumor agents [17].

The low water solubility of pentacyclic triterpenes [18] is a problem for the *in vivo* activity and the bioavailability of OA and UA; a possible pathway in solving this problem is the active compounds complexation with cyclodextrins (CDs). CDs, toroidal-shaped oligosaccharides, are obtained by enzymatic hydrolysis of starch and are characterised by the presence of hydroxyl groups on the outer face of the ring [19]. The physical-chemical and pharmacokinetic properties of the active compounds are substantially improved by CDs complexation because the obtained complexes present a structure with a higher water solubility and in the same time with an important affinity for a great number of hydrophobic substances [20].

In this study OA and UA complexes were prepared using two different CDs: hydroxyl-propyl-beta-cyclodextrin (HPBCD) and hydroxyl-propyl-gamma-cyclodextrin (HPGCD) and kneading as complexation procedure. The products were analysed by differential scanning calorimetry and X ray diffractometry.

2. Materials and methods

OA and UA, at analytical purity level, were purchased from Fluka (Germany). CDs (HPBCD and HPGCD) were obtained from Cyclolab (Hungary).

*Preparation of inclusion complexes.*OA/UA and CDs were kneaded with a 50% ethanol solution in quantities corresponding to a molar ratio of 1:2 until the bulk of solvent evaporated; the obtained mixture was then dried at room temperature for 24 hours and after that it was put into the oven, at 105ºC for several hours. The final product was pulverized and sieved.

*Thermal behaviour.*The DSC analyses were carried out using a Mettler Toledo STAR Thermal Analysis System, DSC 821(Switzerland).

It was used argon as carrier gas, the heating rate was 5 degree/min and the sample weight was 2-5 mg. Examinations were made between 25-300ºC.

*X ray spectra.*The X ray spectra of the six samples were recorded by using a X DRONUM-1 diffractometer (Russia), system with radiation CuKα1 (λ=1.54178 Å) between 2-44° / 2θ. The measurements conditions were the following: target, Cu, filter, Ni; tension, 35kV; current intensity, 20 mA; time constance, 1S; angle $2° < 2\theta < 44º$.

3. Results and Discussions

Thermal behaviour. DSC curve of OA (figure 2a) presents an endothermic peak around the temperature of 117°C indicating the loss of water. The melting point of OA (around 315°C) was not observed in the studied range. The CDs complexes formation is proved on the others two curves (figure 2 b,c) by the peak from 60°C, corresponding to the loss of water and by the disappearance of the peak from 190°C from curve (a).

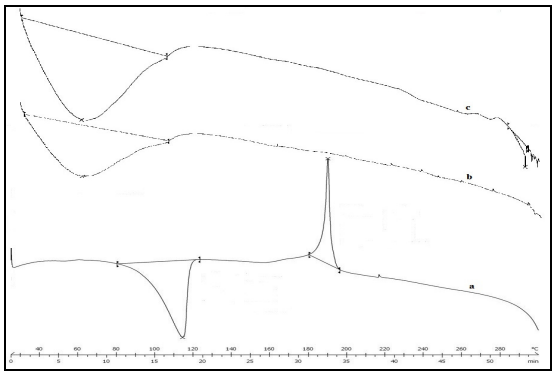

Figure 2. The DSC curves for (a) OA, (b) OA - HPBCD complex, (c) OA - HPGCD complex

In this figure it can be observed that the melting point of the active compound (OA) is not affected (there is no endothermic peaks under 300°C).

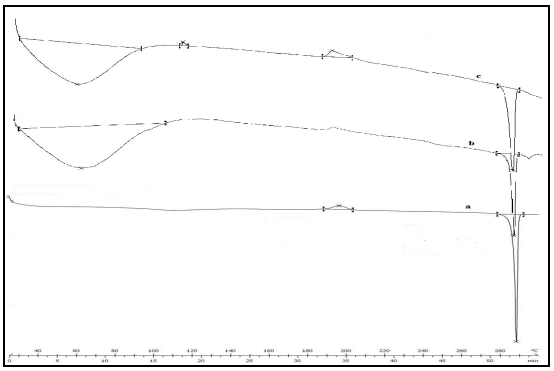

Figure 3. The DSC curves for (a) UA, (b) UA - HPBCD complex, (c) UA - HPGCD complex

The DSC diagram of UA (figure 3a) exhibit an endothermic peak around the temperature of 290ºC, corresponding to the melting point of the substance. Another peak around 195ºC is corresponding to a chemical decomposition of active substance.

The CDs complexes formation is proved on the others two curves by the peak from 60°C, corresponding to the loss of water and it is important to note that the formation of inclusion complexes not changed the peak from 195°C and the melting point of the active substance (from 290°C).

X ray spectra

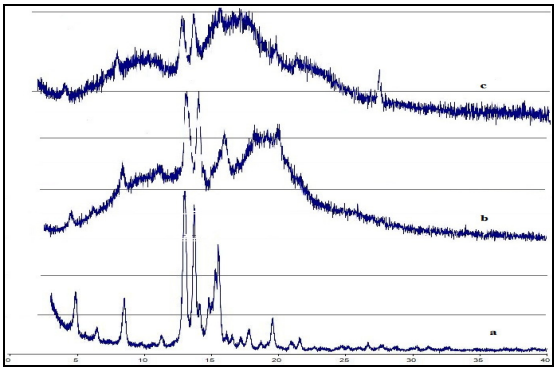

Figure 4. The X ray curves for (a) OA, (b) OA - HPBCD complex, (c) OA - HPGCD complex

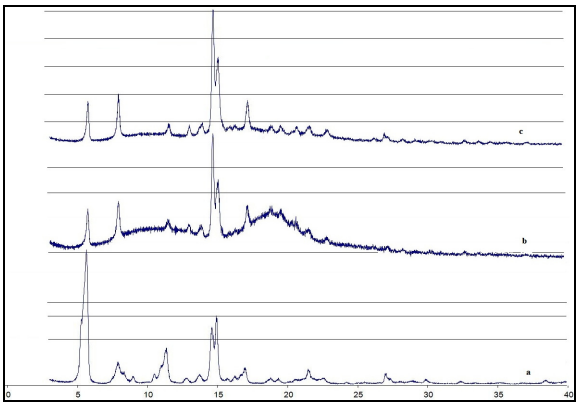

Figure 5. The X ray curves for (a) UA, (b) UA - HPBCD complex, (c) UA - HPGCD complex

X-ray diffraction analyses confirm the previous results. As shown in figure 4 and 5, OA and UA have several peaks characteristic of crystalline compounds, which are virtually absent in CDs inclusion complexes synthesised in this study.

The fact that there is no disappearance of all crystalline peaks leads to the conclusion that an amorphisation phenomenon does not occur, otherwise it can be assumed that inclusion complexes of triterpene substances (OA/UA) and CDs would have a much different spectrum than the spectrum based of OA and UA.

Thermal analyses, particularly DSC curves, present important data about the thermal behaviour of the CDs inclusion complexes. Modifications (disappearance, growth or the diminishing) of the active substance peaks lead to the conclusion that this substance was totally or partially shielded by the CDs ring.

4. Conclusion

Oleanolic acid and ursolic acid are hidroxy-pentacyclic-triterpene compounds of vegetal origin with an important antitumoral activity. Their low water solubility leads to a reduced bioavailability and diminishes the *in vivo* efficacy. The syntheses of inclusion complexes by cyclodextrin complexation can be a simple solution for this problem. The products obtained with HPBCD and HPGCD were analysed by differential scanning calorimetry and X ray diffractometry and it was revealed the complexation between the two acids and cyclodextrins.

The DSC spectra and X ray curves show that were not changed the main characteristics of the active compounds and the cyclodextrins inclusion complexes can be use as drug delivery systems for pentacyclic triterpenes administered *in vivo*.

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