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Original article

The chemical and biological potential of C ring modified triterpenoids

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ABSTRACT

A convenient and elegant route has been developed to separate the natural regioisomers triterpenoids ursolic acid (**UA**) and oleanolic acid (**OA**) as well as derivatives thereof. Eleven unknown derivatives of **OA** were designed, synthesized, and their cytotoxicity was investigated. Further sixteen compounds were prepared to correlate all compounds in a SAR study. It could be shown that C-ring modifications of **OA** and **UA** have only a moderate influence onto the cytotoxic activity of the compounds but a significant impact onto the ability to trigger apoptosis in ovarian cancer cells (cell line A2780).

1. Introduction

Triterpenoids are promising candidates [1] for the development of new drugs. As products of the secondary metabolism they fulfill a function in nature in their own right. But in terms of a future use for medicinal applications, they are far off of being optimized for this use. For these highly complex molecules carrying several stereogenic centers, however, total syntheses are quite challenging but for larger scale synthesis highly uneconomic and libraries of analogs are difficult to obtain. Thus, semi-synthetic strategies are most rewarding; they shorten the time-to-market period tremendously. As a prerequisite, suitable precursors have to be gained from plant material. However, these precursors occur only in small amounts, and, in addition, they are most often part of complex mixtures. Thus, their isolation and/or separation is difficult, sometimes laborious and uneconomic. For their straightforward isolation, extractive steps have to be combined with selective derivatization reactions. The demand for these compounds has increased since clinical trials for NVX-207 [2] or CDDO-Me [3,4] have already begun.

To guarantee the successful commercial exploitation and the development of analogs, effective isolation methods are mandatory and called for. Recently, we were able to present [5] a convenient separation of regioisomeric triterpenoids, ursolic (**UA**) and oleanolic acid (**OA**) on a larger scale. These compounds (Scheme 1) are widely spread in the plant kingdom and known for their broad pharmaceutical activities, *e.g.*, for noteworthy antiviral, antibacterial and anticancer activities [6–8]. However, both molecules occur very often together in leafs or peels of plants [9]. In continuation of our previous work looking for triterpenoids of improved cytotoxic properties, the development of an easy protocol for their separation was still in the focus of our scientific interest.

Several groups were able to show that the introduction of an oxygen substituent into ring C increased the biological activity of the compounds. Due to their ability to act as "reactive oxygen species (ROS) producer", the presence of α,β-unsaturated systems seems promising. For example, for the well-known compound CDDO (2-cyano-3,12-dioxoolean-1,9-dien-28-oic acid) several pharmaceutical activities, *e.g.*, anti-inflammatory, anti-diabetic nephropathy and cytotoxic activities, have been reported [10,11]. Furthermore, clinical trial using ester derivatives of CDDO have been started, thus increasing the demand for this class of compounds [10,12,13]. CDDO esters showed a fast first-pass metabolism; hence, there is still a necessity for improvement [12]. Recently, Leal et al. [14] presented several promising anticancer active lactones possessing an **UA** backbone.

Many data reported for the biological activity of triterpenoids, however, are hardly comparable, and the impact of modifications of the C-ring onto the cytotoxicity of the compounds still remains unclear. Thus, we became interested in studying the structure–activity relationships (SAR) of some of these derivatives combined with a more detailed inspection of their mode of action (MOA).

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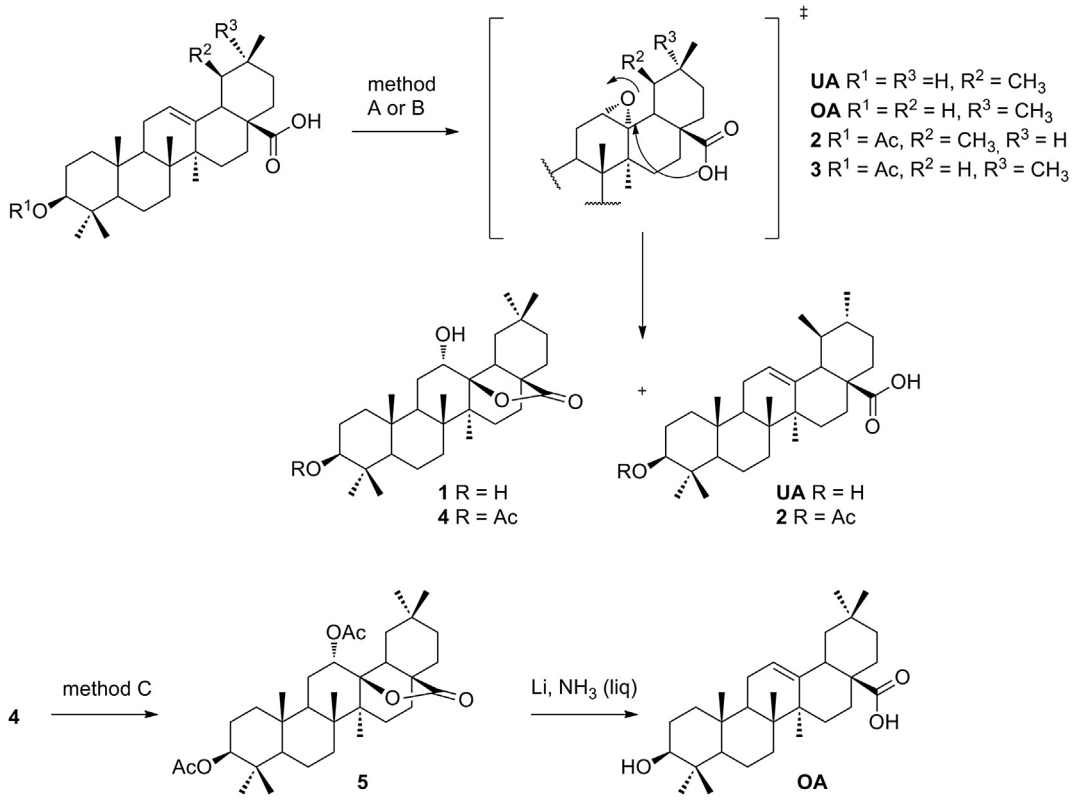

Scheme 1. Separation of a mixture of **OA**/**UA** (and derivatives thereof) and the regeneration of **OA**.

2. Chemistry

Usually the separation of regio- or diastereoisomers can easily be accomplished by chromatography, by recrystallization or by chemo-selective reactions with/without the use of enzymes. Previously, smaller amounts of **UA**/**OA** mixtures have been separated by chromatography [15,16] or by treating these mixtures with HBr [17]. Recently, we presented a new chemo-selective separation of the two constitutional isomers **UA** and **OA** by treating of a **UA**/**OA** mixture with peracids (either performic acid or *m*-chloroperbenzoic acid) [5] under mild conditions. Thus, **OA** is selectively transformed into its corresponding 12-hydroxy-lactone **1** (Scheme 1) while leaving **UA** unchanged. The formation of an epoxide from **UA** is not observed using mild conditions and low temperatures. **UA** is a very valuable starting material [18] for the synthesis of cytotoxic compounds. As early as in 1966, Barton et al. [19] showed that **OA** can be recovered by a reductive elimination (using lithium in ammonia) starting from the 12-acetoxy-lactone **5**. Compound **5** can be prepared either from the 3-hydroxy-lactone **1** by its acetylation with acetic anhydride in pyridine or from the 3-acetoxy-lactone **4**, which was accessed by the separation of a mixture of **2** and **3**. The presence of an additional sharp singulet at $\delta = 2.10$ ppm in the ^{1}H NMR spectrum of **5** indicates – together with an IR vibration band at $\nu = 1766$ cm^{-1} – the formation of **5**. Nevertheless, a low turnover rate of 20% devaluates this separation [19,20]. Consequently, we were looking for a more elegant strategy. Given the potential of C-ring modifications – as exemplified in CDDO-Me – we tried to shorten the synthetic scheme and to optimize the process of extraction and separation.

The treatment of mixtures of ester analogs from **OA**/**UA** mixtures with peracids furnished 12-oxo derivatives from **OA** exclusively but not from **UA**. Thus, **UA** esters remained unchanged under these mild reaction conditions. Moreover, this type of reaction (Scheme 2) could be used for the separation **OA**/**UA** quite universally. Neither the presence of a second double bond in the molecule (as exemplified for **26**) nor the presence of an additional hydroxyl group in ring A (as in **12**) restricted these reactions.

To investigate the influence of C-ring modifications onto the biological activity an oxygen substituent at position 11 (compounds **10** and **17**, Scheme 3) was inserted *via* an α-allylic, chromate-assisted oxidation. For compound **10**, the characteristic IR band for the newly created α,β-unsaturated system was found at $\nu = 1724$ cm^{-1}, in combination with an a signal in the ^{13}C NMR spectrum at $\delta = 200.3$ ppm. Subsequent treatment of **8**, **15** and **24** with selenium dioxide in refluxing 1,4-dioxane resulted in the formation of unknown products. Usually, this type of reaction is known [21–23] to establish a 9,11-ene-12-oxo moiety in steroid-derived structures. However, due to the presence of terminal methyl groups located at positions 26 and 27 in the backbone of the triterpenoids, an elimination of the hydroxyl group at position C-11 is not possible any longer. Hence, a keto group is created instead and rearranged into a thermodynamically more stable enol-system (Scheme 3). For this structural feature, a new signal at $\delta = 142.3$ ppm (C12) is observed in the ^{13}C NMR spectrum. Additional IR vibrations at $\nu = 1652$ and 1614 cm^{-1} confirm the structure [24]. The configuration (Scheme 3) of ring C (compounds **9** and **25**) was ascertained by the shift of the equatorial proton H-1 to higher fields. This shifting (which has already been observed in the past for glycyrrhetinic acid and was also found in compounds **10** and **17**) is caused by an anisotropy tensor of the 11-oxo function [25–27]. Furthermore, cross-peaks in gHMBC NMR experiments between C12 and C18 as well as between C9 and C11 finally confirm this structure which is further ascertained by its ESI-MS spectra ($\Delta m/z = +16$ as compared to the starting material).