Journal of Molecular Structure 1162 (2018) 26–30

Contents lists available at ScienceDirect

Journal of Molecular Structure

journal homepage: http://www.elsevier.com/locate/molstruc

Conformation of flexibly linked triterpene dimers by using RDC-enhanced NMR spectroscopy

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ARTICLE INFO

Article history:
Received 25 October 2017
Received in revised form
31 January 2018
Accepted 21 February 2018
Available online 23 February 2018

Keywords:
RDC-Enhanced NMR
Conformation
Flexible triterpene dimer
MD simulations
Dynamics

ABSTRACT

Dimers of flexibly linked pentacyclic triterpene ursolic acid (UA) and its related frameworks such as asiatic acid (AA) and oleanolic acid (OA) have recently attracted significant attention due to their enhanced anti-cancer and anti-HCV activity compared to their respective monomers. Determination of conformation/inter-monomer orientation of these molecules is very important to understand their structure-activity relationship and to develop new scaffolds, which, however, is difficult through conventional NOE based solution-state NMR spectroscopy, due to lack of long-range NOEs. In the present work, we report a precise determination of conformation of two 1,2,3-triazole-linked triterpene dimer molecules, UA-AA and UA-OA, by employing one-bond C—H residual dipolar couplings (RDCs) as additional long-range orientational restraints, measured in anisotropic PDMS/$CDCl_3$ solvent medium.

1. Introduction

In the recent years, pentacyclic triterpenes based on ursolic acid (UA) [1–3] and its related frameworks asiatic acid (AA) [4,5] and oleanolic acid (OA) [6,7] have gained significant attention because of their anti-cancer activities, viz., inhibition of proliferation and angiogenesis in various cancer cell lines [8]. However, modern therapeutic strategies point to designing molecules, which exhibit multitasking capabilities with improved efficacy. In this regard, it has been shown that chemical modification of UA and its frameworks [9] at C2, C3 and/or C28 positions can enhance their activity, which added further impetus to the development of novel molecules of this class. Amongst these, dimers of same or different UA frameworks linked via 1,2,3-triazole l moiety have gained special focus due to their enhanced anti-cancer and anti-Hepatitis C virus (HCV) activities [10,11], compared to their native monomers. Further, the 1,2,3-triazole moiety [12], is known to exhibit tolerance for both acid and base hydrolysis and is also suitable for selective and efficient cytotoxicity [13,14].

Precise determination of the solution-state NMR structural conformation/inter-monomer orientation of these dimeric molecules: UA-AA triterpene dimer: *1-(3-β-Hydroxy-28-propylcarboxy-urs-12-ene)-4-(2′α,3′β,23′-trihydroxy-28′-methylcarboxy-urs-12′-ene)-1,2,3-triazole* and UA-OA triterpene dimer: *1-(3-β-Hydroxy-28-propylcarboxy-urs-12-ene)-4-(3′β-hydroxy-28′-methylcarboxy-olean-12′-ene)-1,2,3-triazole* molecules is very important to understand their structure-activity relationship as well as to develop analogs with improved target-specificity and activity. However, the task is challenging as the monomer units are well separated (~7 Å) through a non-rigid linker that imparts conformational flexibility to the molecule and also due to the absence of inter-monomer NOEs.

On the other hand, measurement of hetero nuclear one-bond (for example, CH or NH) residual dipolar couplings (RDCs) allows to map the orientation of bond-vectors with respect to the external magnetic field. Unlike NOEs, RDC-based structural correlation (relative orientation of bond-vectors) is not distance dependent, and thereby serves as long-range orientational restraints. Unfortunately, this rich structural information is averaged out to zero in isotropic solvents due to the random tumbling of molecules. However, the RDCs can be reintroduced by dissolving/incubating the study samples in *weakly aligned* solvent media, such as stretched polymer gels and lipid bilayers or liquid crystals. In comparison to the size of the molecules, the pores of the host matrix are expected to be larger by several orders. However, the

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https://doi.org/10.1016/j.molstruc.2018.02.083
0022-2860/

molecules in contact with the surface of the alignment medium (polymer gel matrix) experience *partial* alignment, which imparts anisotropic motion to the molecules and hence non-zero RDCs. The magnitude of the RDCs is proportional to the strength of the alignment medium. The RDCs recorded in weak aqueous alignment media [15,16] have been routinely used in bio-molecular NMR for several years. However, the relatively recent advent of polymer gels that are compatible for *organic solvents* [17], has allowed to measure D_{CH} for synthetic and natural organic molecules as well [18–20]. Importantly, the power of the RDC-enhanced NMR technique has been rightly exploited for unambiguous determination of relative configuration [21–25], conformation [26–31] and hydrogen bond patterns [32] of small molecules including Fibrosterol Sulphate A (steroid dimer) [26]. However, to the best of our knowledge, the technique has not been explored for triterpene dimers. In view of immense pharmaceutical importance of triterpene dimers, here we report the RDC-enhanced NMR spectroscopic conformational elucidation of two hetero dimer molecules of UA-AA triterpene dimer and UA-OA triterpene dimer (Scheme 1).

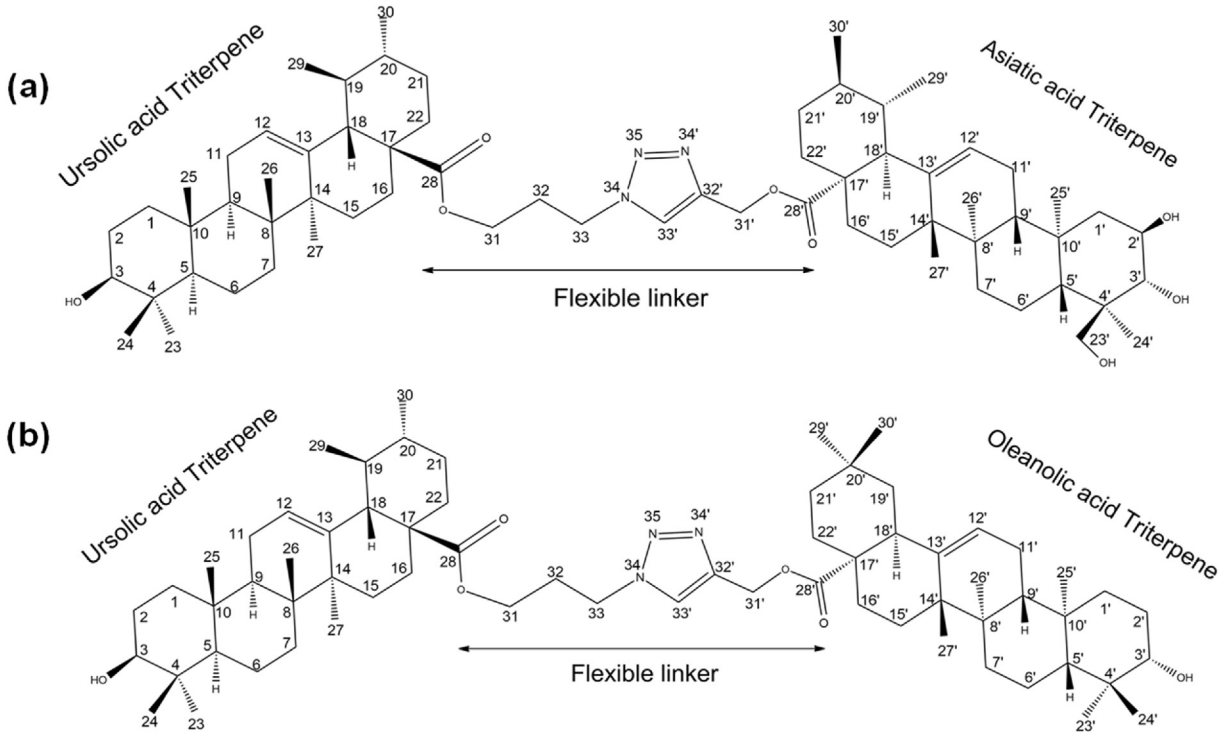
2. Experimental section

The UA-AA triterpene dimer and UA-OA triterpene dimer are synthesized (99% purity) as per the procedure described earlier [10] and confirmed by MASS spectrometry and NMR spectroscopy. All the NMR experiments for the samples dissolved in isotropic and anisotropic media are conducted at 25 °C on 700 MHz Avance-III NMR Bruker spectrometer. About 5 mg of each sample is dissolved in 500 μL of $CDCl_3$. ^1H and ^{13}C resonances are assigned by using a complete set of 1D (^1H, ^{13}C, ^{13}C Distortionless Enhancement by Polarisation Transfer-(DEPT) and 2D $^1H-^1H$ (COrrelation SpectroscopY (COSY), Total COrrelation SpectroscopY (TOCSY), Nuclear Overhauser Effect SpectroscopY (NOESY), $^1H-^{13}C$ (Heteronuclear Single Quantum Coherence (HSQC), Heteronuclear Multiple Quantum Coherence (HMBC) spin correlation experiments (please see the supporting information). One-bond J_{CH} values are measured from [^1H,^{13}C]-CLean In-Phase Heteronuclear Single Quantum Coherence (CLIP-HSQC) spectra recorded in the isotropic $CDCl_3$ solvent. The corresponding residual dipolar couplings (D_{CH}) are measured in anisotropic Poly (dimethylsiloxane) (PDMS)/$CDCl_3$ medium. For estimating the $^1H-^1H$ distance restraints from the NOESY cross-peak intensities, the corresponding integrals are calibrated with respect to the NOESY cross-peak integral for the *geminal* protons at C21, for which the inter-nuclear distance has been taken as 1.78 Å [33].

3. Results and discussions

The ^1H and ^{13}C chemical shift assignments and local configurations of these triterpene dimer molecules, are established in $CDCl_3$ solvent by using the set of 1D and 2D NMR methods as mentioned above (please see the supporting information). These assignments are well correlated with those previously reported for individual triterpene monomers [34] or those linked via ether [35]. It may be noted that the determination of molecular conformation is straight forward for the naturally isolated rigid ether-linked dimers of UA analogs. These compact dimers exhibit well-defined inter-triterpene NOEs that serve as long-range structural restraints [36]. One the other hand, the task is non-trivial for the present molecules. As the linker and the triterpene moieties are connected through a quaternary carbon (C17 or C17′), carbonyl carbon (C28 = O or C28' = O) and oxygen (O) atoms, the sequential and medium-range NOEs between these two parts are not possible. Furthermore, the inter-monomer NOEs are also absent, which can be attributed to the significant separation by the linker ~7 Å and its possible time-averaged conformational changes. Hence, only 12 local NOEs within the triterpene moieties are observed.

Initially, only the NOE-derived distances are used for restrained MD simulations (please see the supporting information). About 100 low energy structures thus derived are overlaid (Fig. 1), which



Scheme 1. Schematic representation of UA-AA triterpene dimer (a) and UA-OA triterpene dimer (b).