Hydrogen Isotope Profile of Methyl Groups in Miliacin (olean-18-en-3βol ME) by Natural Abundance Deuterium 2D-NMR Spectroscopy in liquid crystals

Philippe Berdagué, Philippe Lesot, Jérémy Jacob, Valery T. Terwilliger, Claude Le Milbeau

HYDROGEN ISOTOPE PROFILE OF METHYL GROUPS IN MILIACIN (OLEAN-18-EN-3βOL ME) BY NATURAL ABUNDANCE DEUTERIUM 2D-NMR SPECTROSCOPY IN LIQUID CRYSTALS

Philippe Berdagué[1], Philippe Lesot[1*], Jérémy Jacob[2*], Valery Jane Terwilliger[2,3,4] and Claude LeMilbeau[2]

[1] *RMN en Milieu Orienté (LRMN), Université de Paris-Sud 11, ICMMO, UMR CNRS 8182, Bât. 410, F-91405 Orsay cedex, France.*
[2] *Institut des Sciences de la Terre, ISTO, Université d'Orléans, UMR 7327 CNRS/INSU, BRGM, F-45071, Orléans, France.*
[3] *LE STUDIUM®, Loire Valley Institute for Advanced Studies, 3D Avenue de la Recherche Scientifique F-45071, Orléans, France.*
[4] *Department of Geography, University of Kansas, Lawrence, KS 66045, USA.*
*) Corresponding authors: *philippe.lesot@u-psud.fr; jeremy.jacob@univ-orleans.fr*

Compound-specific hydrogen isotope analyses are gaining increasing interest in paleoenvironmental studies. Numerous works are currently engaged aiming at refining the relationships between the δD of individual compounds and the δD of environmental waters, as well as other climatic and biological parameters. One of the directions proposed is to achieve δD measurements on molecular biomarkers whose source organisms are relatively constrained. Pentacyclic triterpenes are such biomarkers. Because these biomarkers can undergo structural modifications during early diagenesis, it is therefore crucial to understand to which extent these modifications can be accompanied by a hydrogen isotope fractionation process.

As a matter of fact, our previous work on archaeological layers preserved in aqueous conditions in Lake le Bourget revealed that diagenetic by-products of pentacyclic triterpenoids (des-A-triterpenes, aromatic tetra- and pentacyclic derivatives) are systematically D-depleted when compared to their biochemical precursors (Jacob et al., 2011). This depletion can result from: i) the selective degradation of depleted triterpenoids; ii) a microbial or photochemical degradation that is accompanied by fractionation; iii) a heterogeneous distribution of deuterium in the biochemical precursors.

We have tested the later proposition through the analysis of the natural deuterium distribution of methyl groups of a peculiar pentacyclic triterpene, miliacin (olean-18-en-3 β -ol ME or germanicol ME) using deuterium natural abundance two dimensional NMR (NAD 2D-NMR) spectroscopy using polypeptide liquid crystals (PBLG) as oriented NMR solvent. NAD 2D-NMR experiments were carried out on a 14.1 T NMR spectrometer equipped with a

deuterium cryogenic probe (Lesot et al., 2009). This original method, successfully applied for determining the site-specific hydrogen isotopic composition in short-chain, fatty acid methyl esters (Lesot et al., 2008 and 2011), is experimentally extended to the case of methyl groups of miliacin for the first time. Compared to the classical approach using liquid solvents (SNIF-NMR®), the NAD of miliacin signals are spectrally separated on the basis of their chemical shifts and quadrupolar splittings (NMR interaction only observed on spectra recorded in oriented media), thus permitting to advantageously distribute the spectral information on the NMR map. From the evaluation of peak surfaces, it is then possible to determine the (D/H) isotope ratios for each methyl group, when NAD spectra are recorded using quantitative conditions.

The first quantitative results obtained in the case of miliacin isolated from broomcorn millet oil show significant site-specific (D/H) variations at various methyl sites. For instance, methyl sites referred to as Me24 and Me30 are D-depleted compared to Me26 and Me27 while Me31 of methoxy group is significantly enriched in regards of all other sites (see Figure 1). These new results must be regarded at the light of the biosynthetic pathway leading to C3 oxygenated pentacyclic triterpenes through the cyclisation of 2,3-oxidosqualene leading to germanicol, which is finally methylated to provide miliacin. Our results provide clue information on the distribution of D in this type of molecule, thus allowing a better understanding of potential biases in interpreting the δD of the diagenetic derivatives of pentacyclic triterpenes.

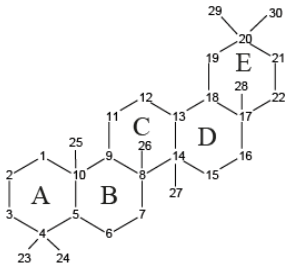

Figure 1. Ring and carbon numbering in oleanane-type triterpenes.

REFERENCES

Jacob, J., LeMilbeau, C., Bossard, N., Disnar, J.R., Billaud, Y., 2011. Combined $\delta^{13}C$ - δD analysis of pentacyclic triterpenes and their derivatives. IMOG 2011, 18-23 September 2011, Interlaken, Switzerland.

Lesot, P., Baillif, V., Billault I., 2008. Combined analysis of four C-18 unsaturated fatty acids using Natural Abundance Deuterium 2D NMR spectroscopy in chiral oriented solvents. Anal. Chem., 80, 2963-2972.

Lesot, P., Courtieu, J., 2009. Natural abundance deuterium NMR spectroscopy: Developments and analytical applications in liquids, liquid crystals and solid phases. Prog. Nucl. Magn. Reson Spectrosc. 55, 128-159.

Lesot, P., Serhan Z, Billault I., 2011. Recent advances in the analysis of the site-specific isotopic fractionation of metabolites such as fatty acids using anisotropic natural abundance 2H NMR spectroscopy: Application on conjugated linolenic methyl esters. Anal. Bioanal. Chem. 399, 1187-1200.

Preferred presentation: “P”