

Miliacin from broomcorn millet – a fossil molecule of multiple interests for archaeologists and paleoenvironmentalists

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Miliacin (olean-18-en-3 β -ol methyl ether) is a fossil molecule that was recently detected in Lake le Bourget (LIB, French Alps) sediments. Comparison of potential plant producers with plants thriving around the lake at time of sedimentation concluded that *Panicum miliaceum* cultivated in the catchment was its most probable source. Since this discovery, it became possible to track the former presence of *P. miliaceum* over its area of extension and diffusion by detecting miliacin in sediments. This first allowed us reconstructing the history of millet cultivation around LIB, characterized by a beginning around 1700 B.C. and a maximum at 800 B.C. before a drastic drop at the Bronze Age/Iron Age transition. Examination of miliacin in sediments from Lake Lledro (Italy) and Paladru (France) revealed distinct dates of its first occurrence, attesting to a complex pattern of diffusion through the Alps. A twin high resolution record of miliacin in LIB Bronze Age sediments evidences periods of low and high concentrations that could result from varying levels of consumption potentially related to climate changes.

While trying to spot paleo-millet fields cultivated during the Bronze Age around LIB, we have analyzed soils in its surroundings. Miliacin is present but not abundant in soils and there is no logical pattern in its spatial distribution. Insufficient miliacin concentrations prevented any ¹⁴C measurement that would have ascertained a molecular legacy of soils from Bronze Age millet cultivation. Reversely, we found miliacin in paleosoils from Ukraine that attested to the in situ cultivation of millet by Iron Age nomad populations.

Miliacin is not solely produced by *P. miliaceum*. Previous studies indicate it can be found in a large spectrum of organisms (gramineae but also, surprisingly, molds), of which *Setaria italica*, the Italian millet. We analyzed seeds of several strains of *P. miliaceum*, several *Panicum* sp. and other gramineae such as *S. italica*. We found miliacin very abundant in any cultivar of *P. miliaceum*, present in all *Panicum* sp., in some *Pennisetum* sp. but absent from three varieties of *S. italica*. In addition, we were unable to recover miliacin from *Chaetomium olivaceum*, a mold, although operating under the

same conditions. A contrary, the mold did not develop, probably due to the reputed anti-fungal properties of this molecule. Incidentally, any bacterial degradation we attempted on miliacin was unsuccessful. Miliacin was only degraded under severe photochemical or acidic stresses.

Miliacin is made of carbon, hydrogen and oxygen isotopes. We determined all isotopic compositions ($\delta^{13}\text{C}$, $\delta^{18}\text{O}$, δD) of miliacin from millet that allowed us confirming (1) it derives from a C-4 plant; (2) its oxygen atom come from atmospheric O_2 , and its δD value allows determining the hydrological conditions that prevailed at time millet grew. This was confirmed by cultivating millet under controlled conditions.

Finally, we purified 6 g of miliacin for proposing it as a standard for the community. We were then able to pierce its hydrogen isotopic intimacy by an innovative Nuclear Magnetic Resonance method. The distribution of deuterium abundances in its methyl groups reflects the biosynthetic pathway of miliacin and molecules affiliated (pentacyclic triterpenes, PT), and allows predicting any isotopic change during the degradation of PT.