Chemical evidence for the preservation of collagen in Eocene turtle shell using Py-GCxGC-TOFMS

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Studies on organic preservation in fossil tissues have been a contentious topic, as fossils have been thought to preserve little, if any, organic content after diagenesis. Several studies have previously reported the presence of collagen in fossils from deep time including in Cretaceous dinosaur bones (e.g.: Schweitzer et al., 2007, *Science* v. 316, 277-280). These findings have also been the subject of criticism with respect to the reproducibility of their results (e.g.: Buckley et al., 2017, *Proceedings of the Royal Society B* v. 284: 20170544). In the present study, we analysed a turtle shell from Eocene to ascertain a suitable proxy for the preservation of collagen, by using comprehensive pyrolysis gas chromatography – time-of-flight mass spectrometry (Py-GCxGC-TOFMS) and comparing the pyrolytic products obtained to those of modern turtle shell and collagen standard.

In order to add to the robustness of the study, industry standards of chitin, melanin and collagen were analysed using Py-GCxGC-TOFMS and their chromatograms compared for characteristic pyrolytic products that can be used to differentiate between them. Collagen could be differentiated from the other nitrogen-bearing biopolymers based on the presence of characteristic cyclic dipeptides known as 2,5-diketopiperazines (DKPs) which are formed by the recombination of peptides during pyrolysis. We compared the chromatogram of collagen standard to that of a modern turtle shell and found that the two chromatograms could be correlated based on the presence of diketodipyrrole, 2,5-DKP(Pro-Pro), 2,5-DKP(Pro-Ala), 2,5-DKP(Pro-Gly), 2,5-DKP(Pro-Hyp), 2,5-DKP(Pro-Arg) and 2,5-DKP(Pro-Lue/Ile). We then compared the chromatogram of modern turtle shell to the Eocene shell and confirmed the presence of diketodipyrrole and 2,5-DKP(Pro-Pro) in the fossil shell thus unambiguously indicating that collagen is preserved.