PATTERNS OF STEROID SYNTHESIS IN MODERN DEMOSPONGES AND THE IDENTIFICATION OF NEW STERANE BIOMARKER TARGETS

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Demosponges are an important source of unusual and bioactive natural products, including a variety of conventional and unconventional steroids possessing complex and different side chain structures. A diverse array of C_{26}-C_{31} sterols have been isolated and characterized from modern demosponge taxa emanating from over four decades of research. This has led to the discovery of well over 100 different sponge steroid structures as well as revealing some robust phylogenetic patterns in the steroid distributions (e.g., Djerassi and Silva, 1991; Giner et al., 1993). Sponge steroids containing unusually alkylated side chains are an attractive choice as a basis for serving as ancient animal biomarkers, especially since demosponges are a derived class of Porifera and some groups of demosponges are known to make a diverse array of unconventional steroids as secondary metabolites. However, first we need to assess suitable sterol targets and determine whether their sterane cores are stable and analytically resolvable from other sterane compounds, as well as other polycyclic biomarker alkanes.

The earliest reported animal biomarkers are from demosponges (Demospongiae) and are recorded in a ca. 100-Myr-long sequence of Neoproterozoic-Cambrian marine sedimentary strata from the Huqf Supergroup (South Oman Salt Basin) commencing in the Cryogenian period (Love et al., 2009; Zumberge et al., 2018). Three different structural series of C_{30} steranes (McCaffrey et al., 1994; Love et al., 2009; Zumberge et al., 2018) are known which occur together in some Cryogenian and Ediacaran (ca. 660-542 Ma) rocks and oils, but which are absent in older samples. In order of chromatographic elution times these are: 24-n-propylcholestane (24-npc), 24-isopropylcholestane (24-ipc) and the newly reported 26-methylstigmastane (26-mes). These steranes correspond with three of the most commonly occurring sterane skeletons in C_{30} sterols found in extant demosponges. The 24-npc steranes found in the Neoproterozoic rock record could possibly be sourced from foraminifera (Grabenstatter et al., 2013), as well as from demosponges (Love et al., 2009). However, the only eukaryotic organisms that are predicted to have the genetic capacity to biosynthesize 24-ipc steranes during the Neoproterozoic era are demosponges, as determined from sterol methyltransferase (SMT) gene sequence analysis combined with molecular clocks (Gold et al., 2016). Given these findings, the co-occurrence of 24-ipc and 26-mes (Zumberge et al., 2018) in the geological record, when 24-ipc/24-npc abundance ratios exceed 0.50 (Love et al., 2009), have been used as strong molecular fossil evidence to track the radiation of the earliest animal life. While future assays may reveal other biological affinities for these molecules, currently demosponges appear to be the most likely Neoproterozoic-Cambrian source of these steranes.

Other than the 24-ipc and 26-mes steranes, no other diagnostic animal molecular biomarkers have been routinely applied to the geological record that are resolvable from the conventional steroids found as abundant membrane lipids of extant microbial eukaryotes. This is perhaps surprising but reflects only an emerging body of knowledge concerning the variety, abundance and taxonomic distributions of unconventional steroids made predominantly or exclusively by animals that can be preserved as detectable and resolvable ancient sterane markers. Other
recalcitrant lipids could expand the molecular biomarker repertoire significantly in the search for early animal fossil evidence. Through the detailed analysis of intact sterol precursors and matching individual sterol precursors to their stable sterane products using mild reduction via continuous-flow catalytic hydropyrolysis (HyPy), we have identified a suite of novel C$_{29}$ to C$_{31}$ steranes that have not yet been reported in the organic geochemical literature which appear to be synthesized exclusively by demosponges. This dual analytical approach has already yielded a more accurate assessment of the variety of demosponge groups capable of producing 26-mes steroids (Zumberge et al., 2018). Our ongoing investigation has revealed at least 6 novel and analytically resolvable sterane structures from lipid analysis of over 90 different species of extant sponges, which could serve as ancient demosponge biomarker targets. In this way, we can considerably expand the existing biomarker repertoire to be able to track the emergence and environmental expansion of earliest animal life on Earth.

References