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Attack of radicals and protons on ladderane lipids: quantum chemical calculations and biological implications†

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Quantum chemical calculations on possible decomposition processes for ladderane lipids are described. Based on the results of these calculations, it is proposed that hydrogen atom abstraction next to the ladderane core can lead to opening of the cyclobutane rings comprising the ladderane substructure, and protonation leads directly to fragmentation. Potential biological implications of these processes are discussed.

Introduction

Despite their geometric strain,[1] ladderane lipids (Fig. 1) are produced in Nature (by so-called anammox bacteria).[2,3] Ladderanes (molecules with linearly concatenated cyclobutane substructures) were synthetic targets long before they were found in Nature due to their seemingly unstable (thermodynamically, at least) hydrocarbon frameworks.[2] They represent yet another surprise from Nature, another "unnatural" molecular architecture that chemists dreamt up only to find that Nature had made it first.[4] While many creative approaches have been applied to the laboratory synthesis of ladderanes,[2] both natural and unnatural, a biosynthetic pathway to the ladderane lipids has not yet been confirmed. In fact, scant few proposals for biosynthetic routes to ladderanes have been published, and the consensus seems to be that unprecedented biosynthetic enzymes are required to form these unusual molecules.[5] One reasonable approach to ladderane biosynthesis would involve the polycyclization of acyclic lipid precursors containing several sites of unsaturation.[2a,5,6] Speculation on the feasibility of several such mechanisms (involving cations, anions and radicals [cation radicals should probably also be considered], "zipping" and "pinching" processes, or perhaps photochemistry) has been published,[2a,5,6] but little firm evidence for the chemical mechanisms involved in biosynthetic ladderane formation exists; a recent report identified genes in ladderane-producing anammox bacteria associated with putative S-adenosylmethionine (SAM) radical utilizing enzymes,[5c] but the nature of the reactions promoted by these enzymes awaits determination.

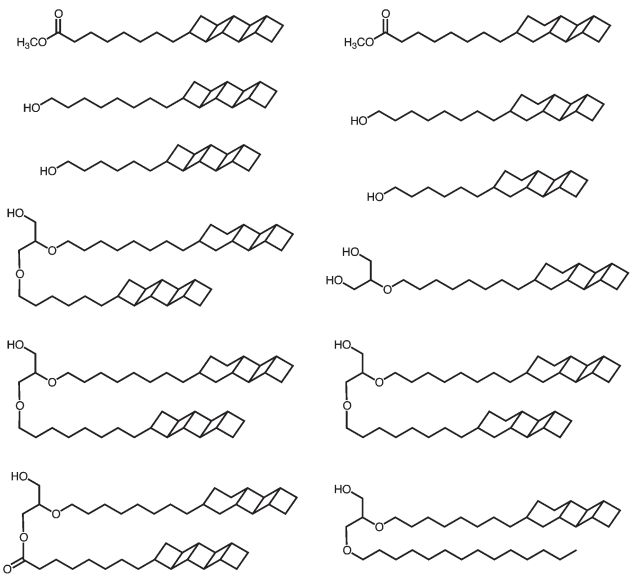

Fig. 1 A selection of ladderane lipids found in Nature.

Results and discussion

Radical unzipping

As part of a study aimed at assessing the viability of polycyclization mechanisms for ladderane formation,[2a,7] we computed (using density functional theory)[8] the energetics for the pathway shown in Scheme 1 (R = CH_3). As drawn, this pathway represents radical promoted ladderane *decomposition*, but we originally considered the reverse reaction corresponding to radical promoted ladderane *formation*. As shown in Fig. 2, this reaction is predicted to be very exothermic (by nearly 40 kcal mol^{-1}; 45 kcal mol^{-1} with CCSD(T)/6-31G(d)//UB3LYP/6-31G(d)[8]) in the ladderane decomposition direction and is therefore very

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†Electronic supplementary information (ESI) available: Coordinates and energies for all computed structures, full GAUSSIAN citations, intrinsic reaction coordinate plots, and additional details on computations. See DOI: 10.1039/c2ob25717c

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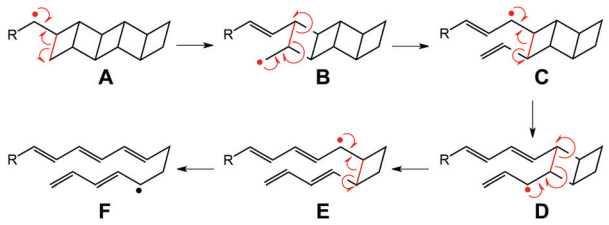
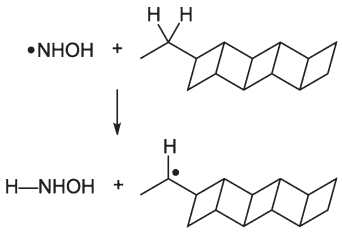


Scheme 1 Proposed mechanism for ladderane fragmentation.



Scheme 2 Model hydrogen atom transfer reaction.

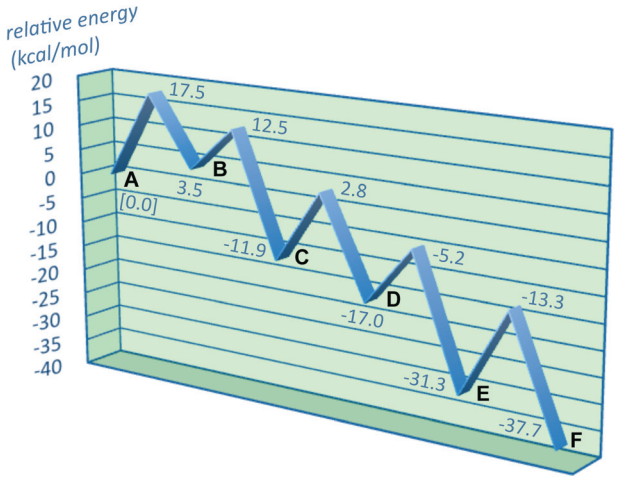


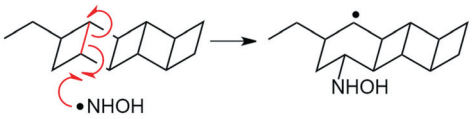
Fig. 2 Computed relative energies (kcal mol^{-1}, relative to that of **A**; UmPW1PW91/6-31G(d)//UB3LYP/6-31G(d)) for species involved in the reaction shown in Scheme 1 (R = CH_3).

endothermic in the ladderane formation direction.[9] Consequently, radical polycyclization of this type (at least if promoted by a single enzyme) is unlikely to be employed for ladderane formation in Nature. Nonetheless, we do think that this type of process may play an important role in the biology of anammox bacteria,[3,5] given that the predicted barrier for fragmentation is low (approximately 17 kcal mol^{-1} with both UB3LYP/6-31G(d) and CCSD(T)/6-31G(d)//UB3LYP/6-31G(d)).

It has been shown that ladderane lipids are major components of the membrane surrounding the anammoxosome, the organelle in anammox bacteria where N_2 is generated from ammonia (or ammonium).[3,5] It has been proposed that this process involves the enzyme catalyzed conversion of nitrite (NO_2^-) to nitric oxide ($NO^{\bullet}$) and/or hydroxylamine (H_2NOH), which is combined with ammonia to form hydrazine (H_2NNH_2), which is then oxidized to N_2.[3,5] During this process, a number of reactive radicals (*e.g.*, O═N–O$^{\bullet}$, $NO^{\bullet}$, $H_2NO^{\bullet}$, $^{\bullet}NHOH$, $^{\bullet}NHNH_2$) might plausibly be formed in the vicinity of ladderane lipids. If any of these small molecules were to diffuse into the membrane, they might well approach the CH_2 group next to the internal cyclobutane ring of a ladderane lipid and, if the energetics allow, abstract one of its hydrogen atoms, thereby initiating the process shown in Scheme 1. But do the energetics allow for hydrogen atom abstraction by these species? HNO and H_2NNH_2 have low bond dissociation energies, approximately 50 and 65 kcal mol^{-1},[10] respectively, indicating that $NO^{\bullet}$ and $^{\bullet}NHNH_2$ are unlikely to readily abstract the necessary hydrogen atom. However, O═N–OH and H_2NOH have higher bond dissociation energies (approximately 75–80 kcal mol^{-1} for each, including both the O–H and N–H bonds of H_2NOH),[10] indicating that these species might more readily abstract a ladderane hydrogen atom. A hydrogen atom on the CH_2 group next to the internal cyclobutane ring might be abstracted, in that the radical produced would benefit from hyperconjugation with the adjacent cyclobutane (bridgehead hydrogen atoms are computed to be more difficult to abstract, but abstraction from a ladderane CH_2 group is predicted to be comparably facile; see ESI†). To probe the feasibility of hydrogen transfer, the model reaction shown in Scheme 2 was examined. Calculations with both UB3LYP/6-31G(d) and CCSD(T)/6-31G(d)//UB3LYP/6-31G(d)[8,11] indicate that this process is endothermic by 19 kcal mol^{-1}. Although this would indicate that there would not be any buildup of the products of this hydrogen transfer reaction, the endothermicity (and barrier for H-transfer, which would be expected to be of a similar magnitude) is not high enough to preclude the formation of some ladderane radicals, which could then fragment. Other more reactive radicals could also conceivably trigger fragmentation (*e.g.*, $ROO^{\cdot}$ or $HO^{\cdot}$, whose H-atom adducts have bond dissociation energies of 87 and 119 kcal mol^{-1}, respectively[10d]).

If this type of reaction does occur, it will damage the anammoxosome membrane. It has been proposed that ladderane lipids make the anammoxosome membrane unusually dense, hindering diffusion of toxic and/or reactive species generated during the N_2 generation process (as well as protons, *vide infra*),[3,5] and it is a reasonable expectation that diffusion will be affected by changes in membrane structure. Although it is not clear at this time how the membrane structure will change upon ladderane fragmentation, the molecular volume of a ladderane lipid increases significantly as it fragments (by >20% overall, even if the two resulting polyene arms are held in an eclipsed conformation).[12]

But is there any potential benefit to an anammox bacterium associated with ladderane unzipping (assuming that the change to the membrane structure is not beneficial)? First, the radical promoted fragmentation may itself prevent reactive radicals from escaping through the anammoxosome membrane, if any are released from enzymes involved in their production; the ladderanes may act as sacrificial gatekeepers, providing a final line of defense against radical release into the greater anammox bacterium. Moreover, the polyenyl systems produced upon fragmentation may also scavenge radicals or other reactive species.[13] In effect, the ladderane lipids may work as antioxidants (or be involved in signaling) in a process not unlike lipid peroxidation[14] that is, here, beneficial to the organism in which it occurs.



Scheme 3 Model radical addition reaction.

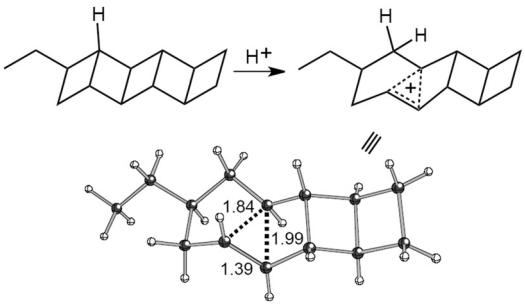


Fig. 3 Model protonation reaction and computed structure (B3LYP/6-31G(d)) of protonated product (distances in Å).

Radical addition

Addition, rather than hydrogen atom abstraction, was also examined. A model addition reaction is shown in Scheme 3. Although this process is predicted to be exothermic by 26 kcal mol^{-1}, it is associated with a barrier of 31 kcal mol^{-1} (UmPW1PW91/6-31G(d)//UB3LYP/6-31G(d)). Consequently, such a process seems unlikely in the absence of a catalyst. Note, however, that if such a reaction did occur, it would generate a [3]-ladderane fused to a cyclohexane, a motif found in some ladderane lipids (Fig. 1).

Protonation

Another process that could convert a [5]-ladderane into a cyclohexane-fused [3]-ladderane is protonation (Fig. 3). The ladderane components of the anammoxosome membrane have been implicated in hindering the diffusion of protons, but whether or not this is merely due to the increased density of ladderane-containing membranes has not been determined.[3,5] An alternative is that the ladderanes themselves actually act as sinks for protons. But is it energetically feasible to protonate a ladderane, which is after all a hydrocarbon? Our calculations suggest that protonation is possible, since it is coupled to fragmentation (Fig. 3), which not only relieves some strain but also leads to a carbocation that is delocalized through 3-center 2-electron bonding, *i.e.*, a nonclassical cation.[15] In fact, the proton affinity of the ladderane shown in Fig. 3 (the energy released upon adding a proton to form the product shown; computed here with B3LYP/6-31G(d)) is computed to be 229 kcal mol^{-1}, a value that is slightly greater than that for trimethyl amine and much greater (by >30 kcal mol^{-1}) than that for a tetrasubstituted alkene or diene.[16] We also examined the addition of hydroxylamine (as a sample nucleophile) to the carbocation formed upon protonation. This reaction is exothermic by 36 kcal mol^{-1} (mPW1PW91/6-31G(d)//B3LYP/6-31G(d)). Thus, not only might [5]-ladderane lipids capture protons, they might then subsequently capture other reactive species, and in doing so, they may be converted to cyclohexane-fused [3]-ladderanes.[17]

Conclusions

Several potential ladderane decomposition processes were described herein and their viability was assessed using quantum chemical calculations. The biological relevance of these processes awaits detailed experimental investigation, but we hope that the results of the calculations reported here will stimulate the design and execution of such experiments.

Acknowledgements

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