Definitive Alkene Identification Needed for In-Vitro Studies with Ole (Olefin Synthesis) Proteins

In their recent article on *in vitro* studies with olefin biosynthesis (Ole) proteins, Frias et al. (1) report the transformation of synthetic 2-myristoylmyristic acid to an alkene (putatively 14-heptacosene) by the combined activities of OleC and OleD. This is one key line of evidence presented to support their proposed alkene biosynthesis pathway. This finding relies solely on GC/MS identification of 14-heptacosene, which was made without reference to an authentic standard; a 70-eV electron-ionization mass spectrum was shown in Figure 6A (1). Based upon that spectrum, we believe that the identification of this compound as heptacosene is open to question. The spectrum does not have the characteristic features of linear, long-chain alkenes (in particular, a weak molecular ion, e.g. 3-15% relative abundance, and *all* of the high-abundance ions in the spectrum occurring as a homologous series between *m/z* ~40-120). This spectrum is dramatically different than representative spectra of the same or closely related alkenes, including heptacosene (Figure 2C in reference 2) and di- and tri-unsaturated heptacosene isomers (Figure 3B in reference 3; composition confirmed by GC-TOF MS) produced *in vivo* by heterologous expression of *oleA(B)CD*, or an authentic 9-hexacosene standard (4). In Figure 6A (1), the presumed molecular ion was uncharacteristically high (100% relative abundance) and there are major, unexpected ions for heptacosene, most notably *m/z* 211 (~45% relative abundance, possibly $C_{14}H_{27}O^{+}$), which is also prominent in the 2-myristoylmyristic acid methyl ester spectrum and in a reported contaminant in that study (1). We believe that re-interpretation of the data is warranted.

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