Gas Chromatography–Mass Spectrometry of Biosynthetic ^{1}H–^{2}H Hybrid Fatty Acid Methyl Esters

By E. OLDFIELD†
(*Department of Chemistry, University of Sheffield, Sheffield* S3 7HF)

Summary ^{2}H enriched lipids have been isolated from the plasma membranes of the organism *Acholeplasma laidlawii* B(PG9) grown on media supplemented with 97 atom% deuteriated lauric acid; g.c.–m.s. of lipid derived methyl esters are interpreted as indicating chain elongation of the *deuteriated* lauric acid by 1 or 2 *protonated* C_2-units.

LAURIC acid was 'perdeuteriated'[1] and contained 97 atom% ^{2}H (by electron impact mass spectrometry). ^{2}H contents were computed using a DCALC program.[2] *Acholeplasma laidlawii* B(PG9) were grown to late log-stationary phase in tryptose broth at 30 °C[3] with tryptose fatty acid depleted[4] and deuteriated lauric acid added to a final concentration of 50 mg l^{-1}. Organisms were harvested by Sharples centrifugation and were burst by osmotic lysis. Freeze-dried membranes were saponified with 1:1 MeOH–2N-aqueous KOH at 30 °C for 20 h. The mixture was acidified and the fatty acids were ether extracted and then methylated with diazomethane.

† *Present address*: Chemistry Department, Indiana University, Bloomington, Indiana 47401.

Myristic and palmitic acids were found to be the predominant fatty acids in the membrane lipids. On rapidly scanning the leading edge of the peak eluting from the gas chromatogram, corresponding to the approximate retention time of methyl myristate, the molecular ion appeared at $m/e = 265$, and on scanning the trailing edge, at $m/e = 242$. The m/e 265 peak was accompanied by a characteristic low

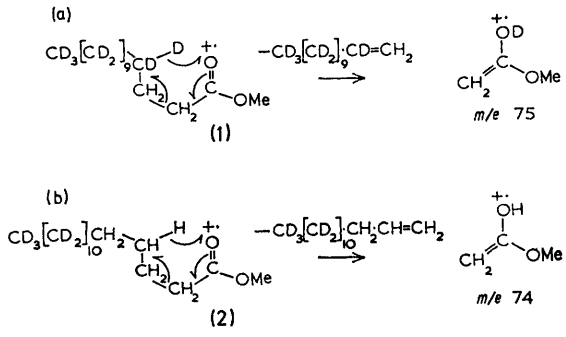

FIGURE. *McLafferty rearrangements in* (*a*) [$^2H_{23}$] *myristic acid, showing* γ-^2H *transfer and* (*b*) *in* [H_{23}] *palmitic acid, showing* γ-^1H *transfer.*

mass number isotopic substitution pattern similar to that observed with the 'perdeuteriated' lauric acid supplement, corresponding to the residual ^1H content of the supplement. It was thus apparent that the $m/e = 265$ ion probably corresponded to $[C^2H_3(C^2H_2)_{10}C^1H_2C^1H_2CO_2Me]^+$ (**1**), indicating that the 'perdeuteriated lauric acid had been elongated by a protonated C_2-unit. Consistent with this interpretation are the known shorter retention times of deuteriated isotopomers, under these conditions, relative to their protonated counterparts.[5]

Similarly, on scanning the leading edge of the peak approximately corresponding to the retention time of methyl palmitate, the molecular ion appeared at $m/e = 293$, and was again accompanied by a characteristic low mass number isotope pattern, and thus likely corresponded to $[C^2H_3(C^2H_2)_{10}C^1H_2C^1H_2C^1H_2C^1H_2CO_2Me]^+$ (**2**). Here, the 'perdeuteriated' lauric acid has been elongated by 2 protonated C_2-units.

The base peak in the mass spectrum of (**1**) occurred at $m/e = 75$, and of (**2**) at $m/e = 74$. This is consistent with the two above proposed structures in which a McLafferty rearrangement,[6] with transfer of a γ-^2H [(**1**), Figure, (a)] or a γ-^1H [(**2**), Figure (b)], occurs. In addition, ions of the general formula $[(CH_2)_nCO_2Me]^+$ with $n = 2$, 6, 10, were apparent in both spectra *e.g.* for $n = 6$, $m/e = 151$ (**1**) corresponds to $[(C^1H_2)_2(C^2H_2)_4CO_2Me]^+$ and $m/e = 147$ (**2**) corresponds to $[(C^1H_2)_4(C^2H_2)_2CO_2Me]^+$.

These findings confirm that chain elongation has occurred from the α-carbon, and are in accord with the findings of Pollack and Toutellotte[7] that *A. laidlawii* B supplemented with shorter chain acids (*e.g.* n-C_6, C_8, and C_{10}) increase production of palmitic acid, by chain elongation.

Utilisation of deuterium labelled precursors together with capillary g.c.–m.s. to resolve and analyse quantitatively isotope hybrid molecules (using *e.g.* AVA techniques) may prove a useful tool on further related biosynthetic studies.

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