Fluorinated fatty acids: new mechanistic probes for desaturases

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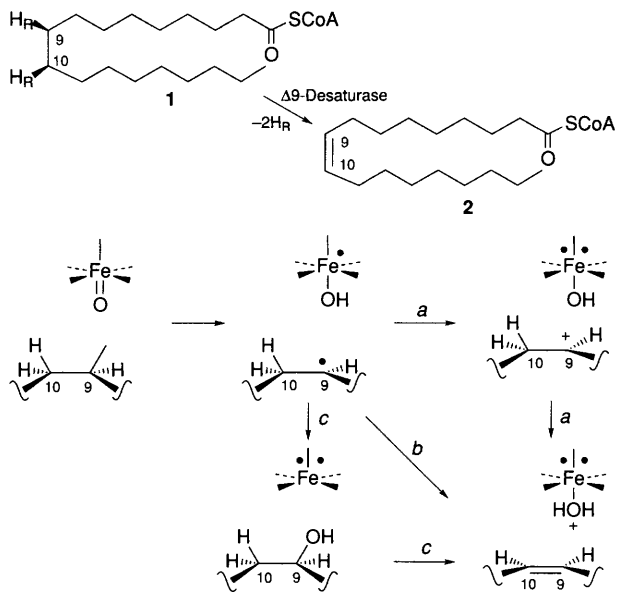
Only (*E*)-fluoroalkenic products are obtained when racemic 9- and 10-fluoro fatty acids are processed by a *Saccharomyces cerevisiae* Δ9 desaturase as determined by ^{1}H-decoupled ^{19}F NMR and GC–MS analysis; no evidence for hydroxylated intermediates was found.

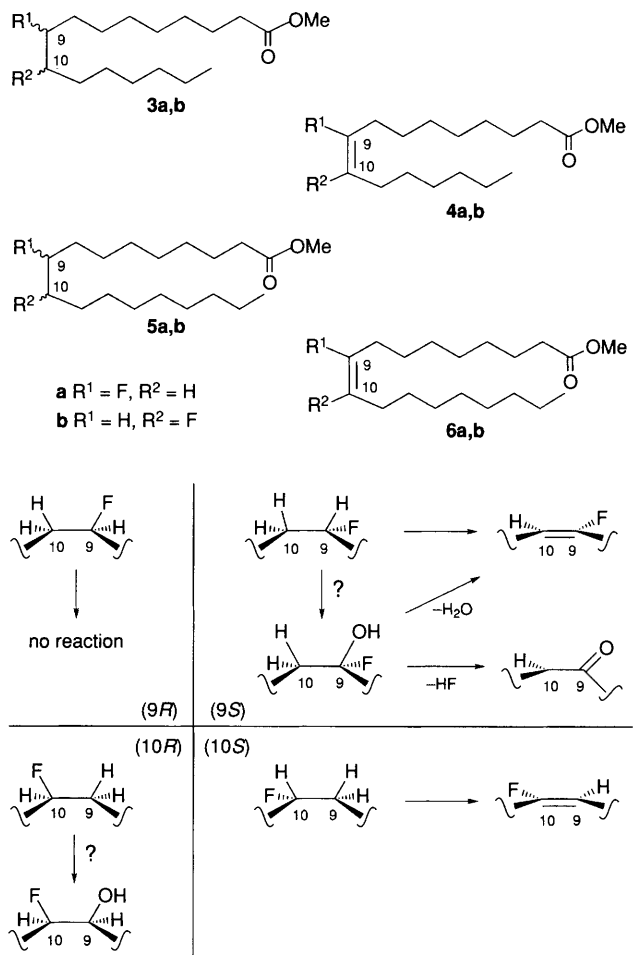
The regio- and stereo-selective introduction of *cis* double bonds into fatty acid hydrocarbon chains is a ubiquitous biological process catalysed by a number of O_2-dependent, non-haem iron-containing enzymes known as desaturases.[1a,b] Interest in the detailed chemical mechanism of these catalysts has grown recently in the context of a biotechnological program to produce modified seed oils.[2] We have obtained results[3,4] using an *in vivo* yeast Δ9 desaturase system, which are consistent with a modified Groves 'hydroxyl-rebound' mechanism involving rate-determining hydrogen atom abstraction to give a carbon-centred radical at C-9 which then collapses to an alkene in one of the three possible ways depicted in Scheme 1.† Very recently, Newcomb and coworkers have re-evaluated evidence for a discrete radical intermediate in both cytochrome P450 and sMMO-mediated hydroxylations and have proposed a 'concerted, nonsynchronous' oxygen insertion mechanism.[5a,b] Given that desaturases and non-haem iron-containing hydroxylases appear to share very similar active site features,[1a,b] the intermediacy of a C-9 hydroxylated species in fatty acid desaturation [pathway (*c*), Scheme 1] merits serious consideration. Here we describe an attempt to use the sterically unobtrusive fluorine substituent as a means of intercepting possible alcohol intermediates formed during desaturation.

Methyl (±)-9-fluoropalmitate **3a**, methyl (±)-10-fluoropalmitate **3b** and the C_{18} homologues **5a,b** were synthesized *de novo*.[6] The substrates were purified by flash chromatography (silica gel, 2% EtOAc–hexane) and all spectral data obtained for these compounds were in accord with their assigned structure. Each fluorinated fatty acid methyl ester (25 mg) was incubated for 24 h with growing cultures (150 ml) of *S. cerevisiae* ATCC 12341 (baker's yeast) as previously described.[3] The yeast cells grew out to a normal extent in each experiment and were collected by centrifugation. The possible fate of the (*S*)- and (*R*)-enantiomers of the 9- and 10-fluorinated substrates is outlined in Scheme 2.‡

The cellular fatty acid fraction from each incubation experiment was isolated by a standard hydrolysis–methylation procedure[4] and analysed by GC–MS[4] and ^{1}H-decoupled ^{19}F NMR.[8] Examination of the fatty acid profiles (Table 1) revealed that high incorporation of the fluorinated substrates was achieved and a single product (a fluoroalkene) was produced in each case. The proposed structure of the fluoroalkenes was consistent with the mass spectral data [**4a,b**: *m/z* 286 (M^+);



Scheme 1



Scheme 2

Table 1 Incubation of fluorinated fatty acids using *S. cerevisiae*

		Cellular fatty acid composition[a]							
Entry	Compound	C_{16}:0	C_{16}:1	C_{18}:0	C_{18}:1	C_n:0(F)	C_n:1(F)	F incorp. (%)[b]	F desat. (%)[c]
1	**3a**	7	35	1	24	29	4	33	12 (10)[d]
2	**3b**	5[e]	30	1	24	28	12[e]	40	—(30)[d]
3	**5a**	6	29	1	20	41	3	44	7 (7)[d]
4	**5b**	7	38	1	16	30	8	38	21 (20)[d]

[a] The relative amounts of each fatty acid component are expressed as a percentage of the total fatty acids and have been determined using the GC–MS Total Ion Current (TIC) chromatograms. Each experiment was carried out twice and each entry represents an average value (SD = 1–2%). Endogenous fatty acids include: C_{16}:0 (methyl hexadecanoate), C_{16}:1 [methyl (*Z*)-hexadec-9-enoate], C_{18}:0 (methyl octadecanaoate) and C_{18}:1 [methyl (*Z*)-octadec-9-enoate]. C_n:0(F) is the saturated, fluorinated substrate, n = 16 (entries 1,2) and 18 (entries 3,4); C_n:1(F) is the unsaturated, fluorinated product, n = 16 (entries 1,2) or 18 (entries 3,4). [b] F incorp. = amount of fluorinated fatty acids as a percentage of the total fatty acids in extract. [c] F desat. = percentage desaturation of fluorinated substrate. [d] Values in brackets were obtained by integration of the relevant peaks in the ^{1}H-decoupled ^{19}F NMR spectra of the fatty acid extracts. [e] These values are based on the ^{19}F NMR data due to overlap of the GC peaks.

6a,b: *m/z* 314 (M^+)], the anticipated GC retention times (*ca.* 0.8 min shorter than that of the corresponding substrate) and the ^{19}F NMR chemical shifts [$\delta_F(CDCl_3, CFCl_3)$ −105.78 (**4a**); −105.57 (**4b**); −105.78 (**6a**); −105.57 (**6b**)]. The (*E*)-stereochemistry of the products was confirmed by comparison of the observed ^{19}F NMR chemical shifts with literature values obtained for (*E*)-7-fluorotetradec-7-ene (δ −105.6),[9] (*Z*)-7-fluorotetradec-7-ene (δ −110.6)[9] and (*E*)-4-fluorooct-4-ene (δ −105.0).[10]

Examination of the CH_2Cl_2 extracts of the culture medium[3] obtained in each experiment revealed only the presence of starting material. None (< 0.1% of the total fatty acids) of the oxygenated diversion products shown in Scheme 2 or their possible metabolites was detected by our analytical methods (GC–MS for non–fluorinated compounds or a combination of GC–MS and ^{1}H-decoupled ^{19}F NMR for fluorinated products).§

The failure to detect methyl 9-oxopalmitate or-stearate or their biological reduction products methyl 9-hydroxypalmitate or-stearate in either the cells or the culture medium of the 9-fluorofatty acid incubations (entries 1,2) is particularly significant. One might have expected that if the 9,9-fluorohydrin was an obligatory intermediate in the desaturation of (9*S*)-fluoro substrates, then some ketone would have been produced (Scheme 2). Biohydroxylation of a CHF moiety to give the corresponding ketone has been documented.[11] We were also unable to observe the production of 9,10-fluorohydrins in our incubations of 10-fluoro fatty acids with the yeast Δ9 desaturase system (entries 3,4). If desaturases operated by a hydroxylation–dehydration route, then (10*R*)-fluorinated fatty acids should have yielded an *erythro*-9,10-fluorohydrin (Scheme 2). On the other hand, if fatty acid desaturation follows a cationic or disproportionation pathway [pathways (*a*) and (*b*), Scheme 1], then (10*R*)-fluoro substrates would be expected to act as suicide inhibitors. That is, the initially formed radical intermediate at C-9 would be forced to attack the enzyme either directly or after formation of the carbocation¶ [pathway (*a*)]. Our experimental approach, which relies on continuous *in vivo* production of Δ9 desaturase, did not permit us to determine whether this enzyme was being inactivated in our incubations using 10-fluorosubstrates. Further work with an *in vitro* desaturase preparation is planned in order to examine this point.

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Footnotes

† The depiction of the oxidant as a monoiron oxo species must be regarded as provisional at this time, since circumstantial evidence indicates there may be two iron atoms at the active site of the yeast Δ9 desaturase.[1b] This uncertainty does not affect the mechanistic considerations presented in this paper.

‡ Since it is known that the yeast Δ9 desaturase abstracts the *pro-R* hydrogens at C-9 and C-10,[7] the fluoroalkenic products can only be derived from the (*S*)-enantiomers present in the racemic substrates.

§ A regioisomeric mixture of methyl *erthyro*-9-fluoro-10- and -10-fluoro-9-hydroxyoctadecanoate [$\delta_F(CDCl_3, CFCl_3)$ −191.48, −191.56], was prepared[12] from *trans*-9,10-epoxyoctadecanoate and used as a reference standard in our search for 1,2-fluorohydrins. The appropriate reference compounds for methyl 9-oxopalmitate or-stearate and methyl 9-hydroxypalmitate or-stearate have been previously synthesized in our laboratory.

¶ The mechanism by which a desaturase might steer the reaction pathway to carbocation formation is currently not known. It is interesting to note that clear evidence for a carbocationic byproduct was obtained in studies[5a] employing cyclopropyl probes and a cytochrome P450 hydroxylating system. A fluorine substituent at C-10 might be expected to raise the redox potential of the putative C-9-centred radical. Whether such an effect would be large enough to prevent carbocation formation is difficult to predict at this time.

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