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FLUORINE SUBSTITUENT EFFECTS ON BIO-METHYLENATION OF OLEFINIC FATTY ACIDS

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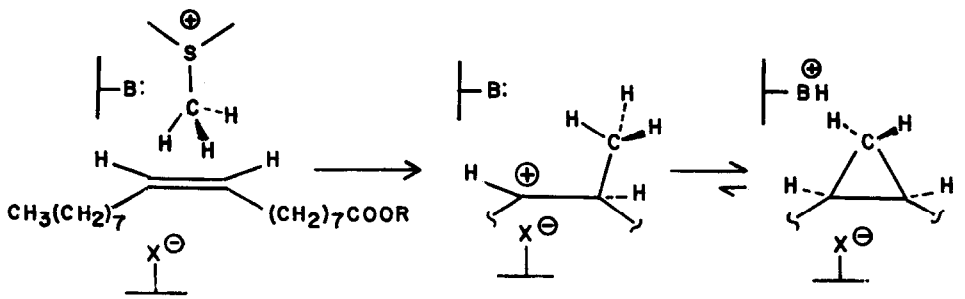
Abstract: A homoallylic fluoro substituent retards the rate of biological methylation of olefinic fatty acids.

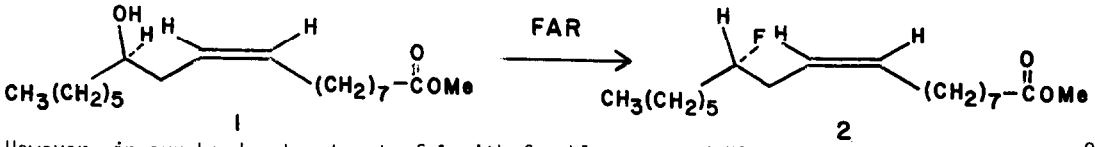
The biological methylation of olefinic cell membrane components represents an important fine-tuning of their physical properties. Two important examples include the methylenation of bacterial olefinic fatty acids[1] and the methylation of phyto- and marine sterols.[2,3] Recently an enzyme active site model for the formation of cyclopropyl fatty acids, by the micro-organism, Lactobacillus plantarum, was proposed[4]; the essential features of this model, as it relates to the methylenation of oleic ((Z)-9-octadecenoic) acid are presented in Scheme 1.



Scheme 1

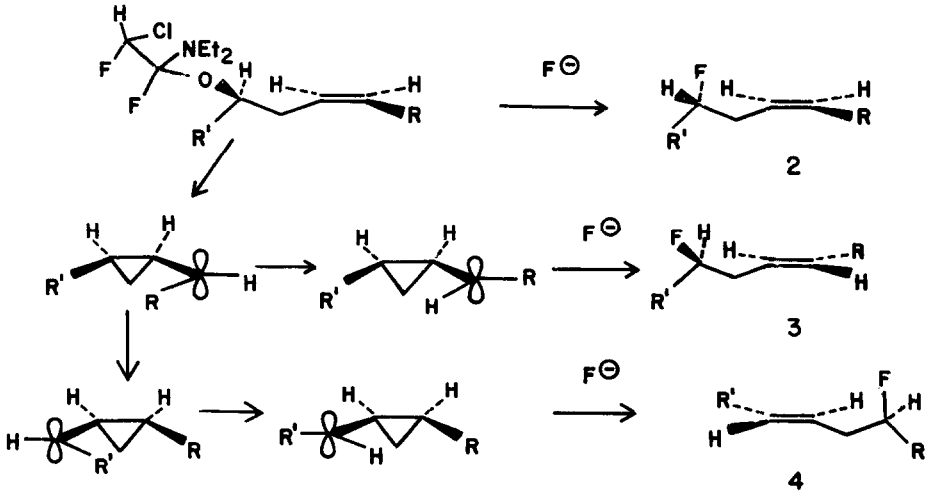
Separation of the sulfonium salt (S-adenosyl-L-methionine) from its counter ion, as first proposed by Akhtar[5], leads to methyl transfer. Reversible collapse of the resultant carbocation by 1,3 proton elimination gives cyclopropyl product. At present, information regarding the nature of the carbocationic intermediate is lacking. It occurred to us that the use of fluorine[6] is an unobstrusive probe for positive charge development might be as helpful here as it has been in other related situations[7]. Specifically, by monitering possible retardations in the rate of biomethylenation upon substitution of fluorine on either side of the olefin, one might be able to deduce the location of positive charge. We wish to report preliminary results which indicate that this approach is indeed feasible.

Few fluorinated olefinic fatty acids have been prepared. As an entry into the problem, fluorination of commercially available ricinoleic([R-(Z)]-12-hydroxy-9-octadecenoic) acid, methyl ester, 1, was attempted. Tosylation of 1, followed by treatment with tetra-n-butylammonium fluoride in refluxing acetonitrile[8] led mainly to elimination products. We then turned to a reported[9] synthesis of methyl 12-fluorooleate, 2, from 1 using the reagent, N-2-chloro-1,1,2-trifluoroethyl-N,N-diethylamine (FAR).[10]



1 2

However, in our hands, treatment of 1 with freshly prepared FAR in methylene chloride at 0°C for fifteen hours, yielded the desired product, 2, along with its trans isomer, 3, and methyl(E)-9-fluoro-11-octadecenoate, 4 in an isolated yield of 70%. Flash chromatography was necessary to remove some starting material. The ratio of 2:3:4 was 0.5:1:1 as determined by ^{13}C NMR and confirmed by a variety of analytical techniques.[11] (The presence of small amounts of the cis isomer of 4 cannot be ruled out.) Scheme 2 portrays how this product mixture might have arisen.



Scheme 2

The desired cis olefin, 2, might arise by direct displacement of the activated alcohol. However, internal attack by olefin can compete to give cyclopropyl carbonium ions which are known to rearrange and collapse to give substituted trans olefins.[12]

Attempts to fractionate this mixture using reverse phase (Whatman, Partisil M9-10, ODS-2, 9.4 mm I.D. x 50 cm) H.P.L.C. were unsuccessful. However feeding experiments using the mixture were still possible. Lactobacillus plantarum can incorporate both cis and trans olefinic fatty acids into their cell membrane during exponential growth phase. As the culture enters the resting state, methylenation of a substantial proportion of cis unsaturated fatty acid phospholipids occurs. Trans olefin is not methylenated[13] presumably because it possesses the wrong configuration for binding to cyclopropane synthetase. Thus the presence of trans fluoro-olefins, would serve only to reduce the concentration of this cis isomer in the cell membrane.

The parameter we wanted to measure was simply the % conversion of olefins to cyclopropyl product as determined by G.L.C. analysis of the fatty acid extract of mature L. plantarum cultures. The results of the feeding experiments[14] are shown in Table 1.

Table 1: Analysis of fatty acids from L. plantarum by G.C.

Exp. #	Compound(s) administered	Fatty Acid Profile* C_{16}:0	C_{18}:0	C_{18}:1	C_{19}:Δ	C_{18}:1(F)	C_{19}Δ(F)	Total olefin+Δ	%Δ formation
1	methyl oleate (40 mg/L)	52.6	1.9	7.2	38.3			45.5	84.0
2	2,3,4 (40 mg/L)	56.1	1.8	-		40.1	1.9**	42.1	4.5
3	2,3,4 (80 mg/L) and methyl oleate (40 mg/L)	51.7	3.6	13.5	19.0	12.2	not detected	44.7	58.5

*The fatty acids were C_{16}:0 (palmitic), C_{18}:0 (stearic), C_{18}:1 (oleate), C_{19}Δ (cyclopropyl oleate), C_{18}:1(F) (fluorooctadecenoate mixture) C_{19}Δ(F) (fluorinated cyclopropyl oleate) as determined by G.C.-M.S.

**C_{19}Δ(F) exhibited ions at m/e 308 (M^+-HF) and m/e 274 (M^+-HF and CH_3OH) in its ms.

The bacteria grew normally (final pH of culture: 4.0) in all three feeding experiments. The first experiment showed that unsubstituted oleate is converted to cyclopropyl product to the extent of 84%. In the second experiment, it was gratifying to see that the fluoroolefins were incorporated efficiently into the cell membrane and that methylenation had taken place, but at a much reduced rate (4.5%). When one corrects for the presence of trans fluoroolefin, for the purposes of comparison with Exp. 1, one can calculate an apparent conversion of 12-fluorooleate to cyclopropyl product of 4.5/0.2 = 20%. This represents a decrease in cyclopropyl fatty acid production of 100 (1.0-20/84) = 76%. We have checked to ensure that this rate retardation is not due to inhibition of the enzyme by trans fluoroolefin. A mixture of our fluoroolefin compounds together with methyl oleate was fed to L. plantarum and as shown in Table 1, methylenation of the cis fluoroolefin could not be detected, but oleate was converted to the extent of 58.5%. Correcting for the presence of trans fluoroolefin and neglecting possible competitive inhibition by cis fluoroolefin, one can calculate an apparent % conversion for oleate of 58.5/(32.5/44.7) = 80.5% in good agreement with the value calculated in Exp. 1 (84.0%).

This study has shown that fluorine induced rate retardations can be observed in our biological system. We are now developing more convenient, general methods for the synthesis of oleates with fluorine on either side of the olefin. The results of feeding experiments using these compounds should enable us to detect the development of positive charge on either C-9 or C-10 or both. The absolute magnitude of the fluorine substituent effect[15] may then also be evaluated in mechanistic terms.

Acknowledgements:

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References and Notes

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11. The structure elucidation of 2,3,4 is based on analytical data on the complete mixture and on partially purified H.P.L.C. fractions:
    2,3,4, MS: m/e 294, (M^+-HF), 262, (M^+-HF, CH_3OH)
    2,3,4, Raman (neat) ν 1670 cm^{-1} (trans C=C), 1657 cm^{-1} (cis C=C) trans/cis ratio 4:1.
    2,3,4, ^{19}F-NMR, ^{1}H coupled, ($CDCl_3$): δ-17.6 ppm from C_6F_6 (m, CHF).
    2,3,4, ^{1}H-NMR ($CDCl_3$): δ 5.45 (m, 2H, vinyl CH), 4.46 (d, J = 45 Hz, 1H, CFH). 2.3 (m, 2H, CHF-CH_2-CH=), 2.02 (m, 2H, HC=CHCH_2) 1.62 (m, 2H, CH_2CH_2CHF).
    2, ^{13}C-NMR($CDCl_3$): δ 27.2 (C-8), 131.7 (C-9), 122.8 (d, J=6.7Hz, C-10), 32.9 (d, J=21.5Hz , C-11) 93.0 (d, J=169 Hz, C-12), 20.5 (d, J=20.5 Hz, C-13). 3, ^{13}C-NMR ($CDCl_3$) δ 33.0(C-8), 133.0, (C-9) 123.5 (d, J=6.3 Hz, C-10), 37.4 (d, J=21.5 Hz, C-11), 93.0 (d, J=169 Hz, C-12), 33.8 (d, J=20.8 Hz, C-13).
    Most of the ^{13}C resonances due to 3 were doublets of nearly equal intensity separated by < .1 ppm, due to the presenceof 4. The ratio of 2,3,4 was estimated to be 0.5:1:1 by ^{13}C-NMR. Von Rudloff oxidation of the mixture followed by G.C.M.S. and capillary G.C. analysis (J. + W. D.B. 5, 0.25μ film) of cleavage products showed that the ratio of positional isomers , 9:11-ene was 57.43 in fairly good agreement with the ratio calculated from the ^{13}C NMR data - namely 60:40. Also the position of the fluorine in each positional isomer was confirmed by G.C.M.S. analysis of the cleavage products.
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