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20-FLUOROMETHYLPYROPHEOPHORBIDE: THE FIRST SYNTHESIS OF A FLUORINE-SUBSTITUTED DERIVATIVE OF CHLOROPHYLL

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Abstract: *Synthesis and properties of the previously unknown 20-F methylpyropheophorbide* **2**, *prepared by electrophilic substitution with cesium fluoroxysulfate* $CsSO_4F$ **5**, *are described. Spectroscopic studies of the influence of the fluorine atom on electronic properties of C-20 substituted chlorins are presented. Properties of* **2** *are compared with those of C-20 H-, Cl-, and Br- substituted derivatives.*

Chlorophyll-containing complexes involved in energy transfer in in vivo and in vitro systems are often difficult to crystallize. Consequently, there is an interest in the synthesis of NMR probes that can be used to study their structures. Such probes must bear a close structural similarity to the chlorophylls themselves; they may then be incorporated into arrays of chlorophyll-protein complexes or chlorophyll aggregates[1,2] without disturbing the structural integrity[3]. Fluorine substitution does not usually perturb the structure of complex organic molecules. Because ^{19}F-NMR is highly sensitive and only an insignificant fluorine background is present in natural biological compounds, fluorinated chlorophylls represent an ideal probe.

The meso positions of porphyrins and the δ location of chlorins are susceptible to electrophilic halogen substitution as has been demonstrated by Woodward and Bonnett[4,5]. Heretofore, fluorine-substitution has been accomplished for porphyrins, but not for chlorins. Balz-Schiemann fluorination of the relatively stable deuteroporphyrin at elevated temperatures has yielded only small quantities of fluorinated product[6]. Recently, Bonnett et al.[7] have used cesium fluoroxysulfate, **5**, to fluorinate octaethyl porphyrin. We have found that this readily prepared reagent[8], a strong oxidant and fluorinating agent with a standard potential of 2.5 V, can be used under controlled conditions to synthesize the C-20 fluoro methyl pyropheophorbide, **2**, (figure 1). We describe the synthesis and then compare the Raman, EPR, and NMR spectroscopic properties of the product with those of the parent compound, as well as with those of the C-20 chlorinated and brominated chlorins[9,10].

Reaction of methyl pyropheophorbide **1** (16 mg, 0.029 mmol, in 16 mL of acetonitrile/methylene chloride, 1/1 v/v) at room temperature with reagent **5** (20 mg, 0.093 mmol, in 12 mL of acetonitrile) added over a period of 20 min and followed by washing with 0.1 N potassium bicarbonate and then H_2O, produced **2** (4.2 mg) with 25% yield. In the process 30% of unreacted material remained, and highly polar decomposition products were formed. An alternate procedure carried out in an ethanol/dry ice bath and with low concentrations of acetonitrile (2.5 % v/v), to avoid precipitation of **1**, (17.5 mg, 0.081 mmol of **5** in 1.5 mL of acetonitrile was added to 30 mg, 0.046 mmol of **1** in 66 mL of methylene chloride over a period of 5 min) yielded similar quantities of product **2** and 50% of the unreacted **1**. Pyrochlorins **3**,

4 were prepared for comparison by halogenation under acidic conditions[11]. The structures and isomeric purity were established by ^{1}H-NMR, ^{252}Cf PDMS and Raman spectroscopy. Compound 2 showed all the ^{1}H-NMR spectral characteristics expected for the C-20 substituted chlorin ring[12]. ^{1}H-NMR of 2 [300 MHz, $CDCl_3$, TMS]: 9.59, 9.58 (s, 1H each, 5-H, 10-H); 8.00 (dd, 1H, 3^1-H_X); 6.29 (dd, 1H, 3^2-H_A); 6.21 (m, 1H, 3^2-H_B; 5.27 (d, 2H, 13^2-H); 4.77 (m, 1H, 18-H); 4.35 (m, 1H, 17-H); 3.70 (q, 2H, 8^1-H); 3.71, 3.60 (s, 3H each, 17^4-H, 12^1-H); 3.53 (d, 3H, 2^1-H); 3.27 (s, 3H, 7^1-H); 2.73-2.19 (m, 4H, 17^1-H, 17^2-H); 1.76 (d, 3H, 18^1-H); 1.69 (t, 3H, 8^2-H). All other structural features of 2 (and also 3, 4) such as the vinyl group, protons at the C-13 carbon, peripheral alkyl groups and two remaining methine protons at C-5 and C-10 carbons were observed[13].

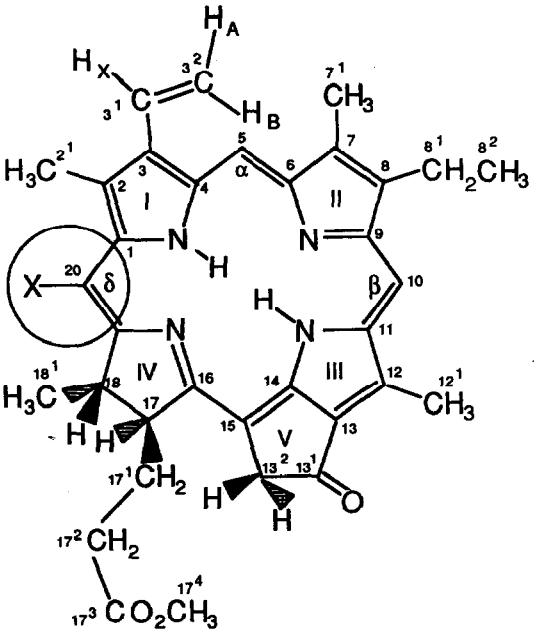

Figure 1. 1: X = H; 2: X = F; 3: X = Cl; 4: X = Br

The ^{19}F-NMR [$CDCl_3$, $CFCl_3$] spectrum showed a singlet at δ -146.20 shifted about 10 ppm upfield with respect to fluorinated octaethylporphyrin[7]. 2D-NMR for the ^{1}H-^{19}F heteronuclear chemical shift-coupling correlation experiment confirmed the proton-fluorine coupling pattern of 2. The projection of fluorine coupling indicates that the doublet at 3.53 ppm (J = 4.5 Hz) can be assigned to the C-2 methyl group and confirms a long range proton-fluorine coupling through five bonds. This doublet assignment provides additional evidence for the chemical shift of the methyl group at C-2^1.

EPR experiments were carried out to assess the effect of halogenation on the high-lying molecular orbitals. The cations of 1, 2, 3 and 4 were prepared either by chemical oxidation using molecular iodine or photochemically using tetranitromethane in benzene as an electron acceptor. Their EPR spectra consisted of single gaussian lines with 10.2, 8.1, 9.4, and 9.1 gauss line width respectively, and no indication of any further structure. The peak-to-peak linewidths are all similar to that of oxidized 1. The EPR linewidth does not change greatly with halogenation, indicating that the half-occupied molecular orbital containing the unpaired electron is not strongly perturbed. Similar experiments in which the triplet state of the halogenated methyl pyropheophorbides were monitored by FT-EPR showed that the zero field splittings changed slightly with halogenation. Electronic spectra of the series showed characteristic bathochromic shifts for the red absorption band of pheophorbides [$CHCl_3$]: methyl pyropheophorbide a < fluoro < chloro < bromo derivatives (666, 669, 673 and 677 nm). The origin of the small shifts in the Soret region is not certain; the 1 nm blue shift of 2 as compared to 1 indicates that no steric or electronic effects are perturbing the structure of 2. Visible spectrum of 2 (in acetone): 408 (vs), 511 (w), 541 (w), 611 (vw), 669 (s); and 4 (in acetone): 410 (vs), 515 (vw), 546 (w), 616 (vw), 677 (s).

Raman spectra showed several frequency shifts and intensity changes when going from 1 to fluorinated, chlorinated and brominated compounds. The frequency shifts are not as pronounced for the fluorinated derivative 2 as for 3 and

4 [14,15]. The band that shows the greatest change is a shoulder at 1606 cm^{-1} for 1 that increases in intensity and shifts to higher frequency by 2-3 cm^{-1} for 2, 3, and 4. The 1500 cm^{-1} band tentatively assigned to $\nu C_a C_m(20)$ is shifted to a higher frequency by 2, 12, and 11 cm^{-1} for the fluorinated, chlorinated and brominated compounds respectively [16]. Electrochemical oxidation potentials have been measured for the halogenated pyropheophorbides vs a standard H_2 electrode. The increase in the redox potential follows in order: methyl pyropheophorbide a < fluoro < chloro < bromo derivatives (0.863, 0.916, 0.938, 0.940 V).

^{252}Cf PDMS mass spectrometry was used to determine the molecular weight of the fluorinated chlorin[17]. A mass shift of 18 amu from 1 confirms the addition of fluorine to the chlorin. An observed molecular cation at m/z 566.3 agrees well with the calculated molecular weight of 566.29 for 2. The protonated molecular ion at m/z 567.6 further supports the mass assignment. The most intense metastable fragment ions of the starting compound, 1, are at m/z 447 and 461, while those of the fluorinated compound are at m/z 465 and 479. From this and other known fragmentation patterns, we conclude that fluorine is not on rings V or IV, which are primary sites for the fragmentation of 1.

The lack of natural background makes C-20 - fluoro substituted pheophorbides attractive ^{19}F-NMR indicators in structural studies. Monofluorinated chlorophylls will be used as NMR probes in studies of chlorophyll aggregates and investigation of chlorophyll complexes with proteins.

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