Synthesis and Properties of 7,7-Difluoro Derivatives of the 2,6-Dioxa[3.1.1]bicycloheptane Ring System Present in Thromboxane A_2

Josef Fried,* E. Ann Hallinan, and Mitchell J. Szwedo, Jr.

Department of Chemistry, The University of Chicago
Chicago, Illinois 60637
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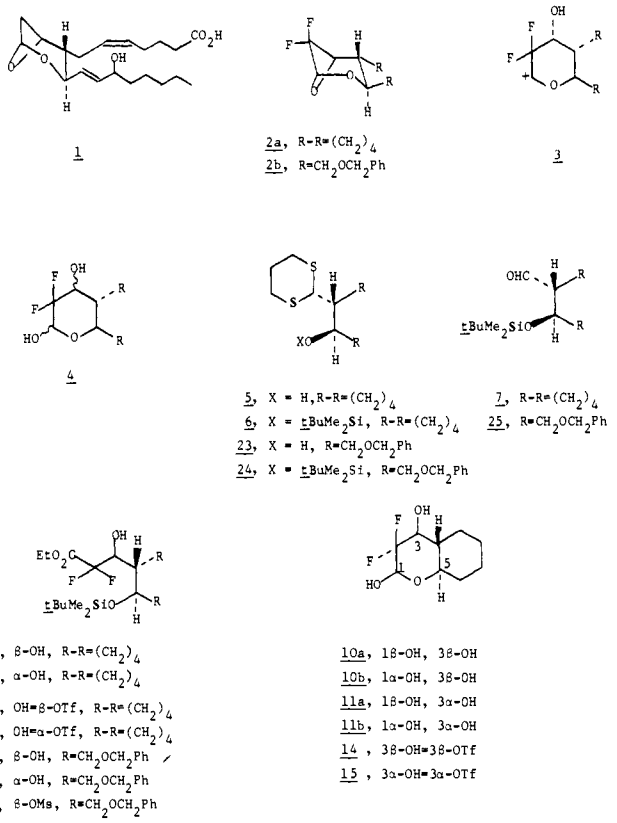
Thromboxane A_2 (TXA_2) **1** the powerful vasoconstricting and platelet aggregating principle derived enzymatically from arachidonic acid is unique among the eicosanoids in that it is not as yet accessible by either total or partial synthesis. One of the reasons for this shortcoming is undoubtedly the lack of stability of this substance to hydrolysis expected of the bicyclic oxetane–oxane[1] structure **1**, which has been proposed for this molecule by Hamberg et al.[2] Because of the important biological properties of TXA_2 a number of more stable analogues of structure **1** have been prepared by substituting carbon and/or sulfur for one or both of the ring oxygen atoms.[3-8] Several of these have shown interesting biological properties.

It occurred to us that the bicyclic oxane–oxetane 2,6-dioxa-[3.1.1]bicycloheptane system could probably be constructed more easily if fluorine were substituted for hydrogen in the oxetane ring α to the acetalic linkage, as, for instance, in compounds of type **2**. Such structures should exhibit great resistance to form the carbonium ion intermediates **3** and thus be resistant to acid hydrolysis. The unusual acid stability of α-fluoro ketals and acetals is well documented.[9] Moreover, substitution of two fluorine atoms for hydrogen in TXA_2 would have a high probability of preserving the characteristic biological properties of TXA_2, as had been demonstrated earlier in the case of 10,10-difluoroprostacyclin.[10-12]

In this communication we describe an efficient synthesis of the ring system **2** and show that its rate of hydrolysis is 10^8 times slower than that of TXA_2. Two structural alternatives were selected, represented by **2a** and **2b**:[13] The basic strategy consists of the synthesis of the difluoro hemiacetals of the general structure **4** of known configuration at the hydroxyl-bearing carbons, followed by conversion of either one of the hydroxyl groups to a leaving group and cyclization to form the oxetane ring. In principle, the procedure introduced by Nerdel et al.[14] for the construction of 2-alkoxy-3,3-dialkyloxetanes from the hemiacetals of 3-(tosyloxy)-2,2-dialkylpropionaldehydes appeared reasonable, since the presence of two fluorines should, as in the dialkyl case, prevent undesired aldol reactions.[15] The required difluoro hemiacetals **10** and **11** were prepared from the protected hydroxy aldehyde **5**.[16] Silylation (*t*-$BuMe_2SiCl$, 1.2 equiv, triethylamine, 1.2 equiv, DMAP, 0.1 equiv in DMF, 16 h at 24 °C, 96%) followed by dethianylation of **6** (added in THF to red HgO, 2.4 equiv, $BF_3{\cdot}Et_2O$, 2.4 equiv in 15% aqueous CH_3CN, quantitative) furnished the aldehyde **7**. Reformatskii reaction with ethyl bromodifluoroacetate[17] in THF (two-step method) yielded the hydroxy ester **8**, mp 55–57 °C, R_f 0.17 (hexane/CH_2Cl_2, 1:1), 37%, and its isomer **9**, oil, R_f 0.28, 17.4%, readily separable by chromatography. The configuration of the hydroxyl group in **8** and **9** was established after conversion of the Reformatskii products into the cyclic hemiacetals **10** and **11** (70%) by reduction with REDAL in ether at −78 °C followed by desilylation (90% CH_3CO_2H, 60 °C, 2 h). In both cases mixtures of the two anomers, **a**, and **b**, were obtained which could be equilibrated by base ($LiN(SiMe_3)_2$ in THF). The stereochemistry at C-1 and C-3 was clearly evident from the proton–fluorine coupling constants seen in both the ^1H and ^{19}F NMR spectra. Thus, diaxial $J_{H,F}$ was 20–21 Hz and diequatorial and axial–equatorial $J_{H,F}$ ranged from 1 to 6 Hz.[18]

The stereochemistry at C-3 in the difluoro esters **8** and **9** now being secure, the esters were converted to the 3β- and 3α-triflates **12** and **13** (Tf_2O, 4 equiv, DMAP, 4 equiv in pyridine, 18 h, 64% and 92%, respectively) and then to the hemiacetals **14** and **15** as mixtures of anomers, as described above for **8** and **9**. When the 3α-triflate **15** was treated with base ($Li(NSiMe_3)_2$, 2 equiv, HMPA, 2 equiv in dry benzene, 6 h at 65 °C) a 50% yield of the oxetane **16** was obtained after chromatography.[19] Methanolysis (0.01 N TsOH in MeOH, 30 min) gave rise to the α-anomer of the 3β-hydroxy methyl glycoside **17**, identical with material prepared by methylation of the 3β-isomer **10a+b** (KOH, ~4 equiv, CH_3I, 8 equiv in Me_2SO, 30 min) followed by chromatography. The isomeric oxetane **2a** could not be obtained by an



(1) The monocyclic oxetane acetal 2-methoxy-3,3-dimethyloxetane, prepared by Nerdel et al. (Nerdel et al. *Chem. Ber.* **1968**, *101*, 1850), was subjected to a careful kinetic analysis of its hydrolysis by Atkinson and Bruice (Atkinson, R. F.; Bruice, T. C. *J. Am. Chem. Soc.* **1974**, *96*, 819) who reported $k = 2.24 \times 10^5\ M^{-1}\ s^{-1}$. From these data a half-life of 78 s is calculated at pH 7.4 and 30 °C. This value is sufficiently close to the value of 32 s at pH 7.4 and 34 °C for TXA_2[2] to lend support to the proposed structure **1**. For additional comparison of 2-methoxy-3,3-dimethyloxetane with TXA_2, see: Fried, J.; Zhou, Z.; Chen, C. K. *Tetrahedron Lett.*, in press.

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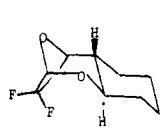

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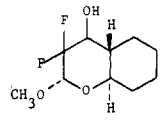

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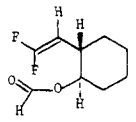

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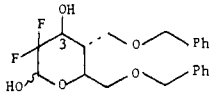
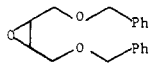


19, 3β-OH
20, 3α-OH
21, 3β-OH=3β-OMs



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analogous procedure from the 3β-triflate **14**. When this latter isomer was subjected to base treatment at 25 °C, or even at −80 °C, rapid fragmentation occurred to form the difluoro olefin formate **18**, whose structure was evident from its NMR spectra. The equatorial orientation of both the 1-hydroxyl (by equilibration) and the 3-trifloxy group is, of course, ideal for Grob fragmentation to occur.

The isomeric oxetane **2a**[19] was obtained as the sole product by converting the axial 3α-isomer **11a+b** into the 1-mesylate (MsCl, 1 equiv, pyridine, 25 °C, exclusively 1β) followed by treatment with NaH (2.5 equiv in benzene, 23 °C, 16 h).

The facility with which fragmentation occurred in the case of the equatorial 3β-triflate **14** to the exclusion of oxetane formation suggested the preparation of the open-chain hemiacetals **19** and **20** in the hope that the β-isomer **19** would undergo oxetane formation via a less-strained transition state. Starting from the readily available *cis*-epoxide **22**[20] and following a series of steps analogous to those described above for the cyclohexane series led smoothly to **19** and **20** as mixtures of anomers. Comparison of their ^{19}F spectra with those of **10** and **11** served to establish the stereochemistry at C-3 and C-1. The 3β-mesylate **21**, mp 119–120.5 °C, was prepared from **26** as the α-anomer via **28** (8 equiv MsCl, pyridine, 20 °C, 3.5 h, 92%) followed by reduction with REDAL in toluene (2 equiv in ether, −80 °C, 75 min) and desilylation (90% acetic acid, 23 °C, 16 h, 71%). In contrast to **14** compound **21** underwent cyclization to form the oxetane **2b**[19] (2 equiv of $LiN(Si(CH_3)_2)_2$, 2.5 equiv of HMPA in DMF, 82 °C, 4 h) in 69% yield after chromatography.

Hydrolysis to the 3α-ol **20** (mixture of anomers) was complete after 8 h in 0.05 N HCl in acetonitrile/water 1:1, establishing the stereochemistry of the oxetane **2b** at C-3 and its formation with inversion at that carbon from the 3β-mesylate. The rate of this reaction was determined at pH 1.27 by using the ^{19}F NMR signals of both **2b** and **20** for following the progress of the reaction. The second-order rate constant of hydrolysis at 23 °C was found to be 2.4×10^{-3} M^{-1} s^{-1} and the half-life of **2b** calculated to be 86 ± 5 min. This compares with $k = 5.5 \times 10^5$ for TXA_2 at 37 °C and a half-life at pH 7.4 of 30 s, a 10^8-fold decrease in rate! Comparing the rate of hydrolysis of **2b** with that of common, unstrained acetals, e.g., diethyl acetal, the former is about 100 times slower.[21] It is evident that the 7,7-difluoro-2,6-dioxa-[3.1.1]bicycloheptane system is sufficiently stable to withstand many chemical operations and to be resistant in biological systems to nonenzymatic chemical change.

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Supplementary Material Available: NMR (^1H, 500 MHz, ^{13}C, 50.3 MHz, and ^{19}F, 188.4 MHz), mass spectrometric, and other analytical data for **2a**, **2b**, **11a**, **6–21**, and **28** (3 pages). Ordering information is given on any current masthead page.

(19) The oxetanes **16** and **2a** are exceedingly volatile. Attempts to obtain them entirely solvent free failed. The yield figures are derived from ^{19}F NMR data. **16**: R_f 0.48 in hexane/CH_2Cl_2, 1:1; ^1H NMR δ 5.59 (dd $J_{H,F}$ = 2.6, $J_{H1,H3}$ = 4.1 Hz, H-1), 4.65 (m, J_{HF} = 6.0 Hz, H-3), 4.14 (m, H-5); ^{19}F NMR δ ($CFCl_3$) 96.2 (dd $J_{F,F}$ = 189.8, $J_{H,F}$ = 2.9 Hz) and 126.7 (dtd $J_{H,F}$ = 2.5, 6.4, 6.4 Hz); mass spectrum, *m/z* 189 (M − 1), 144 (M − HCO_2H). **2a**: ^1H NMR δ 5.52 (d $J_{H1,H3}$ = 4.9 Hz, H-1), 4.58 (dd $J_{H1,H3}$ = 4.9, $J_{H,F}$ = 7.9 Hz, H-3), 3.76 (dt $J_{H,H}$ = 3.7, 9.8, 9.8 Hz, H-5); ^{19}F δ 109.1 (d, $J_{F,F}$ = 176.2 Hz), 136.2 (dd, $J_{H,F}$ = 9.0 Hz); mass spectrum, *m/z* 189 (M - 1), 144 (M − HCO_2H. **2b**: ^1H δ 5.62 (d, $J_{H1,H3}$ = 4.46 Hz, H-1), 4.97 (m, $J_{H3,H1}$ = 4.6, $J_{H3,F}$ = 8.5 Hz, H-3), 3.94 (dt, $J_{H,H}$ = 6.8, 11.1, 11.1 Hz, H-5); ^{19}F NMR δ 109.4 (d, $J_{F,F}$ = 185.1 Hz), 138.4 (dd, J_{Hf} = 8.4 Hz). For difluorooxetane *J* values, see: Bissell, E. R.; Fields D. B. *J. Org. Chem.* **1964**, *29*, 249.

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Surface-Enhanced Raman Spectra of an Active Flavo Enzyme: Glucose Oxidase and Riboflavin Binding Protein on Silver Particles

Robert A. Copeland, Stephen P. A. Fodor, and Thomas G. Spiro*

Department of Chemistry, Princeton University Princeton, New Jersey 08544

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The discovery that adsorption on roughened silver electrodes[1] or silver colloids[2] can increase the Raman scattering cross section of molecules by some 5 orders of magnitude has led to intense interest in the mechanism and applicability of surface-enhanced Raman (SER) spectroscopy. Several studies of complex biological molecules have been published,[3] but for such systems there is a serious question whether adsorption on a metal surface leaves the essential molecular organization intact. We are now able to report a SER spectrum of an active enzyme. Glucose oxidase adsorbed on colloidal silver particles generates a high-quality flavin Raman spectrum (the intrinsic fluorescence being completely quenched) at submicromolar concentrations and shows 86% enzymatic activity while still on the colloid; 95% of the activity is recovered when the enzyme is displaced from the colloid by cyanide.

Figure 1 compares silver colloid SER spectra for glucose oxidase (GO) and riboflavin binding protein (RBP), at 0.58 μM flavin concentration, with the silver-free resonance Raman (RR) spectrum of 0.50 mM RBP. The flavin fluorescence is sufficiently quenched in RBP to permit acquisition of a detailed RR spectrum,[4] whereas GO fluoresces strongly. The GO resonance CARS spectrum has, however, been reported[5] and is similar, except in a few details, to that of RBP. The 488-nm laser excitation is within the first π−π* flavin absorption band[6] and also the large silver particle absorption,[7] centered at 398 nm. Although no internal standard was used in these experiments, the better signal/noise in the 0.58 μM RBP SER spectrum than in the 0.50 mM RR spectrum shows that the surface enhancement produces at least 3 orders of magnitude amplification of the flavin resonance enhancement. This accords with the 10^3 SER enhancement in the

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