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# SYNTHESIS OF A NEW PROSTAGLANDIN ENDOPEROXIDE ($PGH_2$) ANALOG AND ITS FUNCTION AS AN INHIBITOR OF THE BIOSYNTHESIS OF THROMBOXANE $A_2$ ($TBXA_2$)

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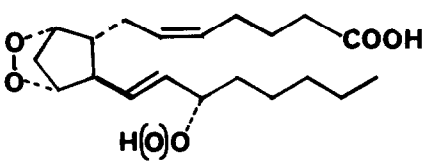
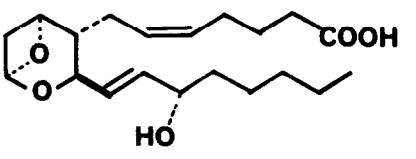
Since the discovery that the prostaglandin endoperoxides $PGG_2$ and $PGH_2$ are converted enzymically to the highly unstable but biologically potent substance thromboxane $A_2$[1-3] by a relatively straightforward mechanism, it has been clear that various synthetic analogs of the PG endoperoxides would be of great interest as possible inhibitors of thromboxane $A_2$ synthetase. Such inhibitors, if both potent and highly selective for $TBXA_2$ synthetase, would be very useful tools for biological research and possibly interesting as antithrombotic agents. A sizeable number of significant papers dealing with both PG and non-PG derivatives as inhibitors of $TBXA_2$ synthetase have already appeared.[4]

Encouraged by our previous experience with the highly active 9,11-azo-bridged analog of $PGH_2$,[5] we settled on the bridged amine 1 as a rational candidate since this substance could serve as a $PGH_2$




$PGH_2 (G_2)$



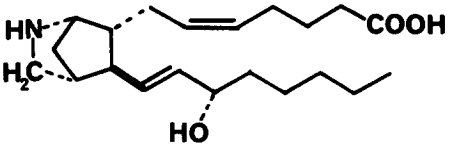

$TBXA_2$

analog with even higher proton (or $TBXA_2$ synthetase) affinity at the 9$\alpha$-heteroatom than $PGH_2$. In addition, 1 was expected to be both readily accessible by synthesis and clearly not susceptible to any of the known $PGH_2$ rearrangements under the proteolytic influence of the $TBXA_2$ synthetase. In this note we describe a simple synthesis of 1 which was developed several months ago and also a more recent study on the inhibitory effects of 1 in $TBXA_2$ biosynthesis. In summary, the biological data indicate that 1 is at least as effective as the reported inhibitors of $TBXA_2$ biosynthesis in human blood platelets and is active at the micromolar level.

The hydroxy keto ester 2 was obtained from the 15-acetate of $PGA_2$ methyl ester by the method previously described,[6] $[\alpha]_D^{25}$ -87.6° (c=1.7 in $CH_3OH$).[7] Selective reduction of the 9-keto function of 2 using sodium borohydride (4 equiv) in ethanol at -30° C for 30 min afforded the corresponding 9$\beta$-hydroxy compound which was isolated in 51% yield after chromatography, $[\alpha]_D^{25}$ -36.1° (c=2.6 in $CH_3OH$).[8] This 9$\beta$-alcohol was quantitatively converted to the bis mesylate-ester by reaction with methanesulfonyl chloride (2.1 equiv) and triethylamine (3 equiv) in methylene chloride at -20° C for 1 hr; $[\alpha]_D^{25}$ -36.0° (c=1.6 in $CH_3OH$), $R_f$ (tlcsg-EtOAc) 0.80. Exposure of this bis mesylate to 0.5 N lithium hydroxide in methanol at 25° C for 4 hr (under argon) provided after isolation 96% of the bis mesylate-acid 3; $[\alpha]_D^{25}$ -12.2° (c=1.7 in $CH_3OH$), $R_f$ (tlcsg-EtOAc) 0.50. Reaction of the mesylate 3 with 40% aq. ammonium hydroxide at 0° C for 5 hr and then 20° C for 48 hr yielded after evaporation in vacuo the ammonium salt of 1. For spectral analysis and characterization 1 was converted to the N-acetyl-15-O-acetyl derivative; $[\alpha]_D^{25}$ -30.0° (c=1.2 in $CH_3OH$); $R_f$ (tlcsg-9:1 $CH_2Cl_2$-$CH_3OH$) 0.45; ir carbonyl max ($CHCl_3$) 1700-1725, 1625 $cm^{-1}$; pmr ($CH_3CON$) 2.03 $\delta$. The methyl ester N-, 15-O-diacetyl derivative of 1 was also characterized; $[\alpha]_D^{25}$ -38.5° (c=0.65 in $CH_3OH$); $R_f$ (tlcsg-EtOAc) 0.5; ir carbonyl max ($CHCl_3$) 1730, 1625 $cm^{-1}$; pmr ($CH_3O$, $CH_3CON$), 3.66 and 2.03 $\delta$.

The evaluation of the endoperoxide analog 1 as an inhibitor of $TBXA_2$ synthetase was carried out relative to two reference substances, imidazole and the 15-desoxy-azo-PGH analog 4 (Upjohn Co., U-51605).[9] Isolated blood platelets (from the blood of non-medicated human volunteers) were suspended in Krebs-Henseleit medium and incubated at 37° C with added $TBXA_2$ inhibitor (at various concentrations) and 1-$^{14}$C-labeled arachidonic acid (New England Nuclear Co.).[10] The incubation mixture was extracted with ethyl acetate and the material so obtained was chromatographed on tlcsg plates using water-saturated hexane-ethyl acetate-acetic acid (5:11:2) for development after addition of non-radioactive $TBXB_2$ as a marker. Iodine vapor located the $TBXB_2$ band which was removed by scraping and counted for $^{14}$C-content. Parallel runs were made with a given batch of platelets for 1, imidazole and 4 under standard conditions so that relative inhibitor potencies could be ascertained. Several batches of platelets gave similar results. Consistently the concentrations of inhibitor required for 50% inhibition of $TBXA_2$ synthetase were far smaller (between $10^{-2}$ and $10^{-3}$ times) for 1 and 4 than for imidazole. The average inhibitory potencies for 1, 4 and imidazole were found to be approximately 830:500:1, respectively.

The strong inhibitory effect shown by 1 on $TBXA_2$ synthetase and its ready availability from $PGA_2$ makes this substance an interesting candidate for further study.[11]

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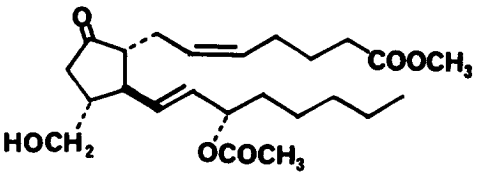


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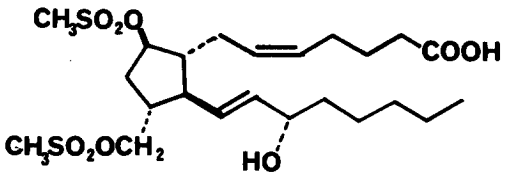


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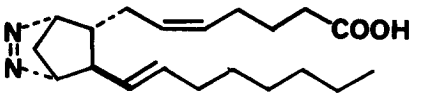


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## References and Notes


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2. B. Samuelsson in "Prostaglandin Research," P. Crabbé, ed., Academic Press, New York, 1977, pp. 17-46.
3. S. Hammarström and P. Falardeau, Proc. Nat. Acad. Sci., U.S., 74, 3691 (1977).
4. See, for example, (a) (imidazole) P. Needleman, A. Raz, J. A. Ferrendelli, and M. Minkes, ibid., 74, 1716 (1977); and S. Moncada, S. Bunting, K. Mullane, P. Thorogood, J. R. Vane, A. Raz, and P. Needleman, Prostaglandins, 13, 611 (1977); (b) (dipyridamol) A. I. Ally, M. S. Manku, D. F. Horrobin, R. O. Morgan, M. Karmazin, and R. A. Karali, ibid., 14, 607 (1977); (c) (N-0164) P. S. Kulkani and K. E. Eakins, ibid., 12, 465 (1976); (d) (burimamide) G. Allen and K. E. Eakins, ibid., 15, 659 (1978); (e) (nicotinic acid) J. E. Vincent and F. J. Zylstra, ibid., 15, 629 (1978); (f) (15-desoxy-9,11-azo-$PGH_2$ analog 4) R. R. Gorman, G. L. Bundy, D. C. Peterson, F. F. Sun, O. V. Miller, and F. A. Fitzpatrick, Proc. Nat. Acad. Sci., U.S., 74, 4007 (1977); and F. A. Fitzpatrick and R. R. Gorman, Biochem. Biophys. Acta, 539, 162 (1978); (g) (15-desoxy-9,11-hydroxylamino-$PGH_2$ analogs) G. L. Bundy and D. C. Peterson, Tetrahedron Lett., 41 (1978); (h) (various) P. Needleman, B. Bryan, A. Wycke, S. D. Bronson, K. Eakins, J. A. Ferrendelli, and M. Minkes, Prostaglandins, 14, 896 (1977).
5. (a) E. J. Corey, K. C. Nicolaou, Y. Machida, C. L. Malsten, and B. Samuelsson, Proc. Nat. Acad. Sci., U.S., 72, 3355 (1975); (b) E. J. Corey, K. Narasaka, and M. Shibasaki, J. Am. Chem. Soc., 98, 6417 (1976).
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7. Satisfactory infrared, proton magnetic resonance and mass spectral data were obtained using purified, chromatographically homogeneous samples.
8. The 9α-epimeric alcohol, $[\alpha]_D^{25}$ -13.1° (c=1.2 in $CH_3OH$) was obtained in 47% yield from this reduction. $R_f$ values for the 9β- and 9α-alcohols were 0.30 and 0.50 (thin layer chromatography-silica gel (tlcsg) with development by ethyl acetate).
9. Sample kindly provided by Dr. G. L. Bundy of the Upjohn Co. to whom we express our thanks.
10. The test inhibitor was first incubated with the platelet suspension for 6 min, then labeled arachidonic acid was added and incubation continued for 10 min after which time the reaction was terminated by addition of 0.2% formic acid in ethyl acetate and the enzymic products recovered by extraction.


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