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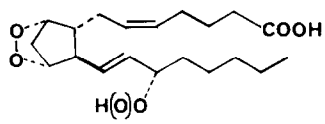
SYNTHESIS OF A NEW SERIES OF POTENT INHIBITORS OF THROMBOXANE A_2 BIOSYNTHESIS

E. J. Corey and Stephen G. Pyne
Department of Chemistry, Harvard University, Cambridge, Massachusetts 02138

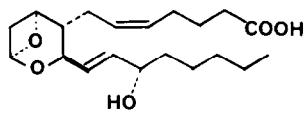
Andrew I. Schafer
Harvard Medical School, Brigham and Women's Hospital, Boston, Massachusetts 02115

Abstract: The pyridino prostanoids 9, 12 and 14 have been synthesized (in racemic form) and have been found to be effective inhibitors of the biosynthesis of thromboxane A_2 in human platelets (IC_{50} 1-3 μM).

Arterial thrombosis plays an important role in a range of pathological conditions including stroke, myocardial infarction and peripheral vascular disease. One possible course of therapeutic intervention lies in the inhibition of the biosynthesis of thromboxane A_2 ($TBXA_2$), the most potent platelet aggregating factor known. Since $TBXA_2$ is formed by enzymic rearrangement of PGH_2 and/or PGG_2, analogs of the endoperoxide precursor, which do not themselves cause platelet aggregation, are of considerable interest. Endoperoxide analogs in which the O(9)-O(11) bridge is replaced by NH-O,[1] $NHCH_2$,[2] and N=N[3] units have been found to inhibit $TBXA_2$ biosynthesis in washed platelets at micromolar concentration, presumably because they mimic PGH_2. Imidazole[4] and pyridine[5] are weak inhibitors of $TBXA_2$



$PGH_2(G_2)$



$TBXA_2$

biosynthesis, perhaps because they are capable of binding to a proton donor associated with the catalytic site. Many monosubstituted imidazoles and pyridines have been tested as $TBXA_2$ biosynthesis inhibitors, some of which show good activity, for example Pfizer UK-37,248-01[6] and Ono OKY-1555.[5] A recent review[7] provides an indication of the intensity with which this area is being investigated.

In view of the inhibitory potency of the 9,11-NH-CH_2 bridge analog of PGH_2 prepared in the laboratory some years ago[2] and the inhibition of $TBXA_2$ synthesis by monosubstituted pyridines[5] it seemed interesting to synthesize and test the pyridine prostanoid 9. We describe herein the results of this study of 9 and also its 5,6,13,14-tetrahydro and 13,14-dihydro derivatives (12 and 14, respectively).

Treatment of 4-lithio-3-bromopyridine 1[8] (prepared from 3-bromopyridine and lithium diisopropylamide, -78°, 10 min) with dimethylformamide (3 equiv, -78°, 1 hr, warming to 25° over 1 hr) gave aldehyde 2 (73%). Exposure of 2 to the sodium salt of dimethyl (2-oxoheptyl) phosphonate in THF

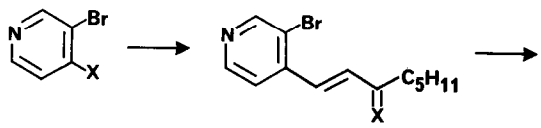
at 25° gave the enone 3 (89%) which upon reduction ($NaBH_4$, EtOH, -30°, 1 hr) gave allylic alcohol 4 (90%) and dihydro 4 (10%). The tetrahydropyranyl ether 5 of 4 was converted to its 3-lithio derivative with t-BuLi (2 equiv, THF, -78°, 30 min) and then to the corresponding organo cuprate derivative (CuCN, 1.1 equiv, -78°, 1 hr) which upon treatment with the iodo allene 10 (1.0 equiv, -78°, 1 hr; -45°, 14 hr)[9] gave the ester 6 (44%). Hydrolysis of the tetrahydropyranyl ether of 6 (CH_3OH, pTsOH, 25°, 12 hr, 87%) gave 7 which upon hydrogenation (1 atm) in benzene at 25° over 5% Pd - $CaCO_3$ (10% by weight) gave 8 (83%). Hydrogenation (1 atm) of 8 in ethyl acetate at 25° over 5% Pd - $CaCO_3$ (50% by weight) gave a mixture containing 11 and 13 in approximately equal proportions which could be separated by HPLC (Waters Associates aminopropyl column, EtOAc/hexane 3:7, retention volumes, 11.5, 12.8 and 15.6 for 11, 13 and 8, respectively). Hydrolysis of 8, 11 and 13 (NaOH, H_2O, EtOH, 25°) gave the acids 9, 12 and 14, respectively.

The pyridine prostanoids were tested as thromboxane biosynthesis inhibitors using washed platelets from human donors who had not taken aspirin or other drugs for at least 10 days. Platelet suspensions were prepared by gel filtration of platelet rich plasma. The amount of $TBXA_2$ biosynthesis from added arachidonic acid (50 μM) was determined by standard radioimmunoassay as previously described.[10] The biosynthetic product was confirmed as $TBXA_2$ by preparative runs using reversed phase HPLC analysis for $TBXB_2$ as follows. Gel filtered platelets were pre-incubated with or without thromboxane synthetase inhibitor for 5 min, and treated with [^{3}H] arachidonate (4 μCi, 10 μM) for 10 min. After acidification to pH 3 and extraction the products were separated by reversed phase HPLC as previously described,[11] using increasing proportions of acetonitrile (30.5 - 95%) in aqueous phosphoric acid (pH 2.0) and compared with authentic standards of thromboxane B_2, prostaglandins, 12-HETE, and arachidonic acid. In the presence of the thromboxane synthetase inhibitor, the production of [^{3}H] $TBXB_2$ was completely abolished, other platelet cyclooxygenase metabolites, [^{3}H] PGE_2, [^{3}H] PGD_2 and [^{3}H] $PGF_{2\alpha}$, were increased, and the platelet lipoxygenase metabolite, [^{3}H] 12-HETE, was unchanged.

Inhibition of $TBXA_2$ synthesis in washed human platelets by pyridino prostanoid 9 (a racemate) was measured over a concentration range of 5×10^{-10} to 5×10^{-3} M. The concentration for 50% inhibition of $TBXA_2$ biosynthesis (IC_{50}) was found to be approximately 3 μM. The tetrahydro and dihydro analogs 12 and 14 were each somewhat more active with IC_{50} of 1 μM.

Inhibition of arachidonate-induced platelet aggregation was also observed with 9, although it was found that concentrations of 9 sufficient to inhibit completely $TBXA_2$ biosynthesis attenuated but did not eliminate aggregation. This discrepancy may be due to accumulation of PGH_2 which is known to cause aggregation.

The activity of the racemic pyridino prostanoids 9, 12 and 14 as inhibitors of $TBXA_2$ biosynthesis is sufficient to warrant further investigation of this series. In addition, it is tempting to consider that these and the simpler monosubstituted pyridines which are active may be functioning as substrate analogs of PGH_2.[12]



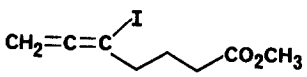
1, X=Li

2, X=CHO

3, X=O

4, X=H,OH

5, X=H,OTHP



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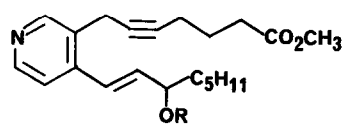
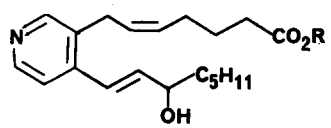


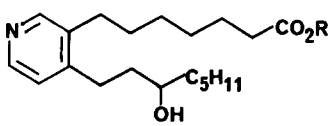
6, R=THP

7, R=H



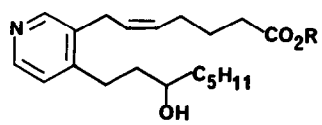
8, R=CH_3

9, R=H



11, R=CH_3

12, R=H



13, R=CH_3

14, R=H

References and Notes

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