Synthesis of 13,14-dehydroprostacyclin methyl ester: A potent inhibitor of platelet aggregation

(prostacyclin biosynthesis/prostaglandin bromo ethers/thromboxane A2/13,14-dehydroprostaglandin F20a)

Josef Fried and Jeffrey Barton

Department of Chemistry and The Ben May Laboratory for Cancer Research, University of Chicago, Chicago, Illinois 60637

ABSTRACT The structure of the most recently discovered, biologically highly active prostaglandin, PG1, or prostacyclin, is correctly predicted on biogenetic grounds, and a general synthesis starting with prostaglandins of the F20 series is reported. Starting with the biologically active 13,14-dehydro-
PGF20, the synthesis involves formation of a 5-bromo-9α-epoxy double bond, followed by esterification and dehydrobromination of the methyl ester to form the prostacyclin structure. The stereochemistry at C-5 and C-6 of all reported products is assigned on the basis of experimental findings and mechanistic reasoning. 13,14-Dehydroprostacyclin methyl ester is considerably more stable at pH 7.5 than prostacyclin. It inhibits platelet aggregation induced by a variety of agents and causes an increase in renal blood flow in the dog at nanomolar levels.

In recent publications, Moncada et al. (1) and Gryglewsky et al. (2) reported the conversion of the prostaglandin (PG) endoperoxides PG2 and PGH2 by pig and rabbit aorta microsomes, to a new unstable prostaglandin, which they named PGX. This substance was characterized by a spectrum of biological activities different from that of all other prostaglandins, notable among which were its powerful inhibition of platelet aggregation and relaxation of arterial strips. Its chemistry was elucidated by Johnson et al. (3) who assigned it to structure I and named it prostacyclin (Fig. 1). It is of more than casual interest that prostacyclin or PG12 (renamed in keeping with well-established usage of capital letters for designating prostaglandins) was also formed by rat stomach fundus microsomes (2), from which Pace-Asciak and Wolfe (4, 5) had previously isolated two substances to which they had assigned structures II and III on the basis of spectroscopic evidence. More recently, Pace-Asciak isolated from the same source a third substance, IV, which is the hemiketalic form of 6-oxo-PGF1a (6). The lack or low level of activity of II in the gerbil colon assay (5) led most observers at the time to consider these substances as curious aberrations of prostaglandin biosynthesis. The discovery of the prominent biological properties of prostacyclin and the finding that it was readily hydrolyzed to the inactive IV served to propel this neglected structural type to center stage of prostaglandin research.

We had long been pondering the question of the significance of cyclic structures of type II, described as early as 1970 by Pace-Asciak, and the mechanism of their biosynthesis from the endoperoxides PG2 or PGH2. It occurred to us that a satisfactory rationalization of the biosynthesis of this structural type could be arrived at by viewing it in conjunction with that of thromboxane A2 (TXA2) (7), which arises from these same precursors. This is shown in Fig. 2: attack by an electrophilic site E+ on the thromboxane synthetase at the oxygen attached to C-9 causes breaking of the peroxide bond and migration of the C-11, C-12 bond to the electron deficient O-11. Product formation then occurs by refolding and attachment of O-9 to the electron deficient C-11 with the liberation of E+. Let us assume now that prostacyclin synthetase is capable of catalyzing a similar sequence of transformations, but now initiates an attack on the oxygen attached to C-11. The electron deficient C-9 oxygen could, as in thromboxane biosynthesis, cause migration of the 8,9-bond (path a); this is a higher energy process, however, because the migrating carbon C-8, in contrast to C-12, is not an allylic carbon. An alternative possibility in this case is an attack on the 5,6-double bond with the formation of a C-6 cation (path b), followed by loss of the C-5 proton to form I. Based on the above view of the parallel nature of the biosynthetic paths leading to both TXA2 and prostacyclin and considering the short half-life at 22 °C reported for the latter (1), we formed the working hypothesis that one of the two enol-ether structures represented by I was most likely candidate for the structure of prostacyclin.

During recent years, this laboratory has been intensely interested in a group of prostaglandin analogs, the 13-dehydro-
prostaglandins, in which the trans-double bond is replaced by a triple bond (8, 9). Several members of this class have been prepared by total synthesis and their biological properties examined. For the purposes of this discussion, it may suffice to state that its prototype, 13,14-dehydro-PGF2a, (V, Fig. 3) behaves biologically like a classical F prostaglandin and possesses all the properties of the latter that have been examined. It therefore seems appropriate to synthesize 13,14-dehydro-
prostacyclin, and to examine its biological properties. In the following, we describe a rational synthesis of the Z-isomer of I.

EXPERIMENTAL

General Conditions. Nuclear magnetic resonance (NMR) spectra were obtained on a Bruker HX-270 spectrometer (270 MHz) in CDCl3 using tetramethylsilane for calibration. Chemical shifts are given in δ and coupling constants in hertz;

This mechanism differs fundamentally from that proposed in ref. 5 in that it does not require O2.

1 This synthetic procedure was presented at the annual symposium of the Intra-Science Research Foundation in Santa Monica, CA. December 1–3, 1976.
FIG. 1. Structures of prostacyclin and prostacyclin metabolites.

multiplicities: s (singlet), d (doublet), t (triplet), q (quartet), and m (multiplet). Infrared (IR) spectra were recorded on a Perkin Elmer 137 spectrometer in CHCl₃. Spectra were calibrated against the polystyrene 6.238 μm absorption. Rotations were measured on a Perkin Elmer model 141 polarimeter using a 10-cm path length in CHCl₃. Mass spectra were recorded on a Finnigan 1015 quadrupole spectrometer equipped with a vpc inlet; spectra were collected and processed by a Systems Industries Computer Interface System/150 and are expressed as percentage of the base peak (in parentheses). Microanalyses were performed by Baron Consulting Co., Orange, CT.

(5R,6R)-9-Decoxy-5-bromo-6,9α-epoxy-13,14-dehydro-PGF₁α (1a) and Its (5S,6S) Isomer (1b). To a solution of 0.203 g (0.58 mM) of 13,14-dehydro-PGF₂α, in 20 ml of dry acetonitrile was added 0.115 g (0.65 mM) of N-bromosuccinimide. The solution was stirred at room temperature until thin-layer chromatography (TLC) showed disappearance of starting material (2-4 hr), and then the solution was concentrated to dryness under a stream of nitrogen. The residue was washed with four 2-ml portions of 5% NaHCO₃, the combined aqueous fractions were extracted with CH₂Cl₂ (3 × 2 ml), acidified with 2 M HCl, and again extracted with CH₂Cl₂ (4 × 2 ml). This last extract was dried over MgSO₄, concentrated, and chromatographed on 18.6 g of silica gel (1 × 56 cm column, washed with CH₂Cl₂). Elution was carried out with 2% MeOH/HOAc/H₂O (90:10:6.5, vol/vol) in CH₂Cl₂ in 5-ml fractions.

Fractions 5–8 (1a-15-ketone, 20 mg): IR (CH₂Cl₂): 2.85 and 2.94 (OH), 3.1–4.1 (COOH), 4.51 (C=O), 5.80 (COOH), 5.97 (CO). NMR: 4.68 (m, 1H, H-9), 4.28 (m, 1H, H-6), 4.21 (q, 1H, H-11), 3.99 (m, 1H, H-5), 2.68 ([t(6), 1H], 2.54 ([t(7), 2H], H-16), 2.49–1.22 (m, 17H), 0.89 (t, 3H, H-20).

Fractions 13–18 (1b, 50 mg): NMR 4.51 (m, 1H, H-9), 4.35 ([t(7), 1H, H-15], 4.22 (m, 1H, H-6), 4.03 (m, 1H, H-11), 3.83 (m, 1H, H-5), 2.83 (m, 1H), 2.51–1.2 (m, 20H), 0.92 (t, 3H, H-20). m/e (bis-TMS ether TMS ester): very similar to that of 1a shown below (TMS is the abbreviation for trimethylsilyl).

Fractions 19–45 (1a, 180 mg): NMR 4.63 [t(7), 3, 1H, H-9], 4.34 [t(7, 2), 1H, H-15], 4.24 (m, 1H, H-6), 4.07 [q(7), 1H, H-11], 4.00 (m, 1H, H-5), 2.72 (m, 1H, H-10β), 2.51 [t(8), 1H, 2.43 (m + t(7), 1 + 2H, H-2), 1.2–2.2 (m, 15H), 0.93 (t, 3H, H-20). m/e (bis-TMS ether TMS ester): 575/577 (1, M-C₃H₁₁), 405 (1, 575-577-HBr-TMSOH), 395/397 (1, 575-577-2TMSOH), 395 [2, M-CHBr(CH₂)₃CO₂TMS], 305 (2, 395-TMSOH), 215 (6, 305-TMSOH), 459/461 (2, 575-577-C₃H₆(TMS)), 379 (1, 459-461-HBr), 289 (1, 379-TMSOH), 217 [10, 289-(CH₃)₂Si=CH₂].

1a-Acetate (Ac₂O/pyridine, 25°): NMR: 5.53 [t(7, 2), 1H, H-15], 4.98 [q(7), 1H, H-11], 4.62 [t(7, 2), 1H, H-9], 4.22 (m, 1H, H-6), 3.94 (m, 1H, H-5), 2.63–2.8 (m, 2H), 2.49 (m, 3H), 2.05 (s, 3H, OOCCH₃), 2.04 (s, 3H, OOCCH₃), 1.2–2.1 (m, 20H), 0.86 (t, 3H, H-20).

The above acids 1a and 1b were treated separately with a slight excess of diazomethane (0.5%, Et₂O/MEOH, vol/vol 2:1), then concentrated under reduced pressure to give the corresponding methyl esters. 1a-Methyl ester, 164 mg, 64% yield from 13,14-dehydro-PGF₂α(V). [α]D₅ = +27.1° (c, 1.0 in CHCl₃).

Analysis: Calculated for C₃₃H₃₆O₄Br: C, 56.88; H, 7.22; Br, 17.85. Found: C, 56.66; H, 7.51; Br, 18.13. 1b-Methyl ester, 44.9 mg, 17.5% yield from V. The mass spectra of the bis-TMS ethers methyl esters of 1a and 1b were very similar. m/e derived from 1b: 588/589 (1, M⁺), 573/575 (2, M-C₂H₅), 557/559 (0.5, M-OCH₃), 499/500 (5, M-TMSOH), 517/519 (89, M-C₃H₇), 427/429 (8, M-C₃H₇-TMSOH), 437 (6, 517-519-HBr), 347 (13, 437-TMSOH), 275 (16, 347-Me₂Si=CH₂), 508 (6, M-HBr), 421

FIG. 2. Proposed mechanism for the biosynthesis of thromboxane A₂ and prostacyclin from PGH₂. E⁺, enzyme possessing an electrophilic site. (A) Thromboxane A₂. (B) Prostacyclin. The enantiomeric (ent) structures are used in B in order to simplify the presentation. a = path a; b = path b.
Fig. 3. Conversion of 13,14-dehydro-PGF$_2\alpha$ into 13,14-dehydroprostacyclin and related products. The compound designation next to the arrow indicates which compound was used in that particular reaction.

[10, 508-(CH$_2$)$_2$CO$_2$CH$_3$], 331 (10, 421-TMSOH), 241 (23, 421-2TMSOH), 395 [4, M-CHBr(CH$_2$)$_3$CO$_2$CH$_3$], 305 (3, 395-TMSOH), 215 (6, 395-2TMSOH), 351 (2, 395-CH$_2$=CHOH), 261 (5, 395-TMSOH), 279 (2, 395-C$_2$H$_5$TMSOH), 189 (6, 279-TMSOH). $lb$-Methyl ester was converted into the 11,15-bismethoxymethyl ether with $\alpha$-chlorodimethyl ether and diethylamine at 25° for 18 hr. Workup was with hexane/ethyl acetate (4:1, vol/vol) and 2 M HCl. NMR: 4.92, 4.57 (d, 7), 1H each, OCH$_3$O), 4.71, 4.64 (d, 8), 1H each, OCH$_2$O), 4.39 (m, 1H, H-9), 4.31 (t, 6), 1H, H-15), 4.07 (q, 7), 1H, H-11), 3.98 (m, 2H, H-5, H-6), 3.67 (s, 3H, CO$_2$CH$_3$), 3.36 (s, 6H, OCH$_3$), 2.89-3.61 (2H), 2.36 (t, 6), 2H, H-2), 2.44-1.22 (16H), 0.90 (t, 3H, H-20). m/e: 493/441 (0.7, M-OCH$_2$CH$_2$OCH$_2$OH), 369/371 (5, 493/441-C$_2$H$_5$), 339 (2, M-CH$_3$(CH$_2$)$_2$CO$_2$CH$_3$), 277 (3), 215 (10), 297 (3, 339-CH$_3$=CHOH), 255 (3), 173 (20), 189 (2, 339-C$_2$H$_5$).  227 (5), 251 (2, 339-CH$_3$=CHOH)OCH$_3$), 189 (48).

(5S,6R)-9-Deoxy-5-hydroxy-6,9a-epoxy-13,14-dehydro-PGF$_2\alpha$ 1 → 5 Lactone (2a). A mixture of 15.4 mg (0.036 mM) of the bromo ether acid 1a and 23.7 mg (0.16 mM) of 1,5-diazabicyclo[5.4.0]undec-1-ene (DBU) was placed under nitrogen and diluted with 0.1 ml of toluene. The mixture was then heated at 58-60° for 2 hr, cooled, diluted with toluene, and washed rapidly with 0.5 ml 2 M HCl. The toluene extract was filtered through Na$_2$SO$_4$ and concentrated under a nitrogen stream to give 4.6 mg of pale yellow oil. IR: 2.91 (OH), 5.80 (lactone CO). NMR: 4.57 (m, 1H, H-9), 4.38 (t, 6), 1H, H-15), 4.29 (m, 1H, H-5), 4.15-4.05 (m, 2H, H-6, H-11), 3.24 (m, 1H), 2.78-1.26 (m, 20H), 0.92 (t, 3H, H-20). m/e (bis-TMS ether): 494 (2, M$^+$), 479 (3, M-CH$_3$), 423 (100, M-C$_2$H$_5$), 333 (83, 423-TMSOH), 243 (60, 423-2 TMSOH), 395 [52, M-CH$_2$(CH$_2$)$_2$COO$^-$], 305 (69, 395-TMSOH), 215 (100, 395-2 TMSOH), 325 (13, 395-C$_2$H$_5$), 11.15-Diacetate of 2a. NMR: 4.35 (td, 7, 2), 1H, H-15), 5.01 (q, 6.5), 1H, H-11), 4.58 (m, 1H, H-9), 4.26 (m, 1H, H-5), 4.12 (m, 1H, H-6), 2.81-2.63 (m, 2H), 2.63-2.42 (m, 3H), 2.09 (s, 3H, COCH$_3$), 2.07 (s, 3H, COCH$_3$), 2.22-1.24 (m, 15H), 0.90 (t, 3H).

Methanalysis of 2a with 1% TsOH in MeOH for 2 hr at 25° gave the ester; m/e [tris-TMS ether methyl ester]: 598 (2, M$^+$), 583 (6, M-CH$_3$), 493 (7, 583-TMSOH), 403 (12, 583-2TMSOH), 567 (7, M-OCH$_3$), 477 (4, 567-TMSOH), 387 (17, 567-2TMSOH), 508 (27, M-TMSOH), 418 (13, M-2TMSOH), 328 (32, M-3TMSOH), 497 (5, M-CH$_3$(CO$_2$CH$_3$), 407 (4), 317 (11), 469 (8, M-CO$_2$CH$_3$-C$_2$H$_5$), 379 (4), 527 (10, 11, C$_2$H$_5$). 437
(31), 347 (11), 395 (>100), 305 (>100), 215 (>100). 2a-Methyl ester triacetate: NMR: 5.54 (t(t7), 1H, H-15), 4.97 (m, 2H, H-5, H-11), 4.49 (m, 1H, H-9), 4.11 (m, 1H, H-6), 3.66 (s, 3H, COCH3), 2.78–1.22 (m, 20 H), 2.41 [t(t7), 2H, H-2], 2.07 (s, 6H, COCH3), 2.05 (s, 3H, COCH3), 0.89 (t, 3H, H-20).

(5R,6S)-9-Decoxy-5-hydroxy-6,8a-epoxy-13,14-dehydro-PGF1α - 5 Lactone (2b). A mixture of 2.7 mg (0.006 mM) of bromo acid 1b and 5.1 mg (0.033 mM) of DBU was heated as described above for 2a. Extraction with 0.5 ml of M HCl followed by work up of the toluene extract gave 1.1 mg of yellow oil. NMR: 4.48 (m, 1H, H-9), 4.34 [t(t6), 1H, H-15], 4.3 (m, 1H, H-5), 4.21 (m, 1H, H-11), 3.84 (m, 1H, H-6), 2.76 (s, 2H), 2.69–1.18 (m, 18H), 0.87 (t, 3H, H-20), 11.15-Diacetate of 2b. NMR: 5.32 [t(t6, 2), 1H, H-15], 5.08 [q(t6), 1H, H-11], 4.63 [t(t5), 1H, H-9], 4.47 [t(t6, 3), 1H, H-5], 3.83 [d(t9, 5), 1H, H-6].

Acid Hydrolysis of (5Z)-9-Decoxy-6,8a-epoxy-13,14-dehydro-PGF2α Methyl Ester (13-Dehydroprostacyclin Methyl Ester, 3). Solutions were prepared by dissolving 0.1-mg samples of 3 in 40 μl of acetonitrile, and then adding 10 μl of an appropriate aqueous acid solution at 25°. Aliquots were examined by TLC (EtOAc/silica gel) at various times. Using HClO4 (0.01 or 0.001 M) or 1 M HOAc as the acidic medium, we noted complete disappearance of 3 and appearance of 6-keto-13-dehydro-PGF2α methyl ester after 2 min. Using 0.1 M HClO4 or 0.1 M HOAc, we noted ca 50% hydrolysis after 2 min and complete disappearance after several hours.

The hydrolyzed samples were evaporated, treated with MeONH2-HCl in pyridine, and then examined by gas chromatography/mass spectroscopy: m/e 612 (2, M-C8H17), 537 (2, M-TMSOH), 447 (5), 596 (2, M-OCH3), 556 (2, M-C8H17), 466 (8), 376 (15), 506 (35, 596-TMSOH), 416 (80), 512 (2, M-C8H17CO2CH3), 425 (2), 332 (8), 390 (55, 442-CH3OH).

Stability of 13,14-Dehydroprostacyclin Methyl Ester (3). A 1-mg sample of 3 was dissolved in 4:1 (vol/vol) mixture of 0.1 M Tris buffer, pH 7.52–95% EtOH. Samples were removed periodically and examined by TLC (EtOAc/silica gel). No significant change was observed over a 20-hr period at room temperature.

RESULTS AND DISCUSSION

The synthesis described in this paper mimics the proposed biosynthesis of prostacyclin in that it achieves formation of the tetrahydrofurany ring by reaction of the 5,6-double bond with the 9α-hydroxyl group. The relevant intermediates as well as derivatives essential for proof of structure are shown in Fig. 2. Thus, treatment of 13,14-dehydro-PGF2α, with N-bromosuccinimide in acetonitrile yielded a mixture of bromoesters 1a and 1b in a 4:1 ratio, which were readily separated by chromatography from each other and from a small amount of the 15-keto of 1a. Neither of the isomeric bromides showed NMR signals for the vinyl protons at C-5 and C-6 present in the starting material. Instead, there appeared two new multiplets at 4.00 and 4.24 δ (major isomer) and 3.83 and 4.22 δ, indicating methine protons at these same carbons compatible with the proposed bromo ether structures. The fact that 1a and 1b are 5- rather than 6-membered ethers was evident from the mass spectra of their bis-trimethylsilyl ether trimethylsilyl esters and bis-trimethylsilyl ether methyl esters, all of which showed an ion at mass 395 indicating cleavage between C-5 and C-6 with loss of C-1 to C-5. This structural assignment was confirmed after conversion of the acids 1a and 1b to two isomeric δ-lactones 2a and 2b on treatment with DBU in toluene, each of which likewise formed an ion at 395, in this case as one of the major peaks. Formation of the lactones occurs by displacement of bromide by carboxylate anion with inversion at C-5. Their 11,15-diacetates, which showed the five low-field methine protons as separate NMR resonances, provided structural confirmation.

The assignment of structures 1a and 1b to the major and minor isomers, respectively, is not a trivial matter. Certainly, trans-addition to the cis-double bond would be expected to occur giving rise to two threeo structures. An examination of molecular models predicts a less crowded transition state for 1a, which on this basis should represent the major product. This argument appears to be in contradiction to the results obtained when the methyl esters of 1a and 1b were subjected to dehy-
drobromination with DBU in toluene at 100°C, which constitutes the second and final step of our synthesis. In this reaction, the major product 1a methyl ester afforded the enol ether 3(13,14-dehydroprostacyclin) in 97% yield, so formulated because of the appearance of a single NMR signal at 4.21 δ characteristic of vinyl ether protons, coupled with the disappearance of the methine resonances for H-5 and H-6, and a strong enol ether absorption at 6.09 μ in the IR. The mass spectrum shows several bands confirming this structure, notably a strong ion at 421 indicating loss of a fragment corresponding to C-1 to C-3. Hydrolysis of the enol ether 3 was complete after 5 min in 2 mM HClO₄ in acetonitrile/water (4:1 vol/vol) at 25°C, and yielded the expected 6-keto ester. The product was identified by conversion into the methoxime and comparison of its mass spectrum with that reported for the methoxime of 6-keto PGF₁₀₂ methyl ester (6). The formulation of 3 as the Z ether is based on the premise that trans elimination of HBr constitutes the preferred course of the reaction. Reaction of the minor bromo ether 1b with DBU afforded a double-bond isomer of 3 to which structure 4 is assigned because of the presence of two trans vinyl protons at 5.52 and 5.72 δ coupled on one side to a methine and on the other to two methylene protons. It is this difference in the dehydrobromination reaction of the two bromo ethers that appeared, at first sight, to be at variance with the assignments of structures 1a and 1b: the 6α (endo) methane proton of the former should prove less accessible to the base than the 6β (exo)-proton of 1b; that is, 1a should give rise to 4 and 1b to 3.

There is, however, another interpretation of the results of the dehydrobromination reaction that is in accord with our previous assignment. If, instead of attack by the external base, intramolecular deprotonation occurred via the 11α hydroxylate anion, the proximity of the latter to the 4-methylene protons of 1b could, by preferred abstraction of the 4-pro-S proton, give rise to 4, whereas in the case of 1a that proton would be inaccessible to the internal base. To test this hypothesis, we converted 1b into the 11,15-bismethoxymethyl ether and subjected the latter to reaction with DBU. In this case, the 11,15-bis ether of 3 was formed by attack of the external base on the 6β-proton. This experiment provides evidence in favor of intramolecular proton abstraction as long as a free 11α-hydroxyl group is available. It also reveals the interesting fact that intramolecular abstraction of the 4-proton is preferred over bimolecular formation of the enol ether by abstraction of the more readily accessible 6β-proton.

The half-life of natural prostacyclin in Tris buffer at pH 7.6 is approximately 10 min (3); this is most unusual for an enol ether. We have examined the stability of the methyl ester 3 under these same conditions and found it to be essentially unchanged over a 24-hr period. This radical difference is not likely to be due to the presence of the 13,14-triple bond but rather to that of the methyl ester grouping. One may speculate that internal delivery of the carboxyl proton present in equilibrium at pH 7.6 might be responsible for this considerable enhancement in the rate of hydrolysis of free prostacyclin.

13,14-Dehydroprostacyclin methyl ester (3) was examined (C. L. Malmsten, S. Hammarstrom, J. Fried, and J. Barton, unpublished data) for its capacity to inhibit aggregation of human platelets induced by a variety of agents (7) and was found to possess potency similar to that reported for natural prostacyclin (3). Thus, the inhibitory dose (ID₅₀) was 0.5 ng/ml when induced with arachidonic acid, 2.5 ng/ml with ADP; 1.2 ng/ml with collagen, and 8 ng with PGE₂. When compared with PGE₂, 3 was 10 times more potent as an inhibitor of ADP-induced aggregation. Compound 3 was also tested (T. Paustian, J. Fried, B. M. Chapnick, L. P. Seigen, A. L. Hyman, and P. J. Kadowitz, unpublished data) for its ability to affect renal blood flow in anesthetized dogs (10). Dose levels of 3 low enough to attain a concentration of 1 ng/ml in renal arterial blood produced a 20% increase in renal blood flow.

The levels of biological activities reported here for compound 3 suggest that it possesses the characteristic structural features of prostacyclin. Published data (3) support structure I for prostacyclin, which lacks definition only as far as the stereochemistry of the double bond is concerned. The synthetic route described in this paper defines 3 as possessing the Z structure. We therefore favor the Z isomer of I as representing the structure of prostacyclin.

The authors wish to thank Prof. B. Samuelsson and Dr. C. L. Malmsten of the Karolinska Institute for the platelet aggregation assays and Prof. P. J. Kadowitz of Tulane University for the renal blood flow experiments. This work was supported by National Institutes of Health Research Grant AM-11499, RCA-AM-21846, and Diabetes Center Grant AM-17046. Support by National Science Foundation (GP-33116) and National Institutes of Health Cancer Center Grant CA-14509 to purchase the NMR equipment used in this work is likewise acknowledged.

The costs of publication of this article were defrayed in part by the payment of page charges from funds made available to support the research which is the subject of the article. This article must therefore be hereby marked "advertisement" in accordance with 18 U. S. C. §1734 solely to indicate this fact.