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High-Field ^{1}H NMR Studies of Prostaglandin H_2 and Its Decomposition Pathways

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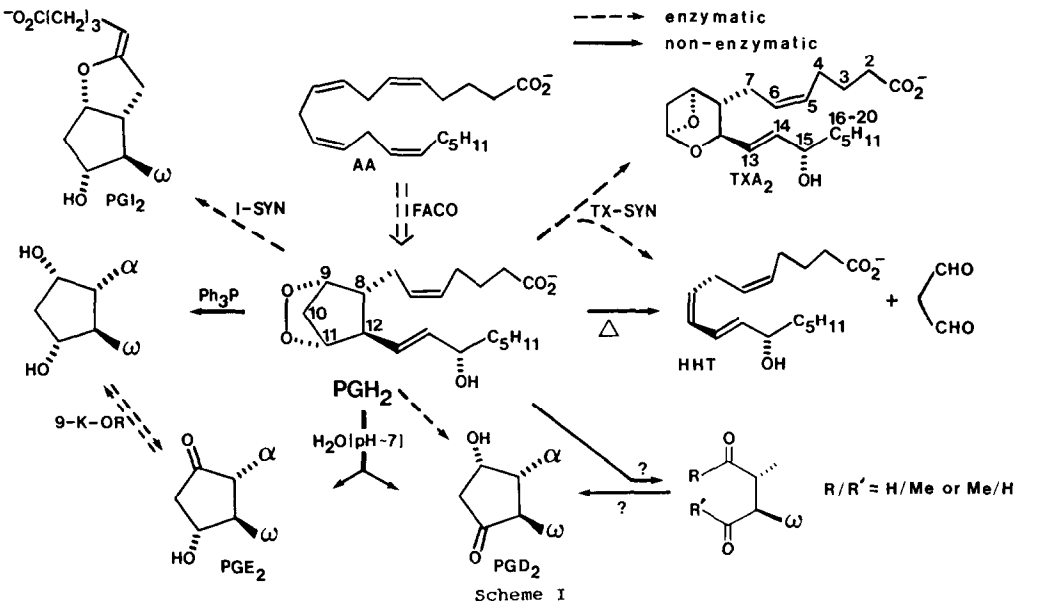
SUMMARY: Prostaglandin H_2 displays at 500 MHz a detailed ^{1}H-NMR in which all methylene groups are non-equivalent in C_6D_6 solution. The spectrum was assigned by analogy to isosteric structures. The dissymmetric perturbation and steric hindrance of the bicyclo [2.2.1] core caused by the side-chains provides a rationale for the selective fragmentations which PGH_2 undergoes. Purified PGH_2 is considerably more robust than previous literature accounts suggest. The following transformations were monitored by ^{1}H-NMR: 1) O-O bond cleavage by Ph_3P, 2) aqueous media fragmentation to PGE_2 and PGD_2, 3) base catalyzed fragmentation to ketoaldehydes, and 4) thermolysis attempts.

The prostaglandin endoperoxide (PGH_2) plays a central role in arachidonate metabolism: as a product of fatty acid cyclo-oxygenase (FACO) (1) and as substrate for enzymes producing a variety of prostanoids, Scheme I. Enzymatic pathways (2) and thermal decomposition (2,3) are reported to afford the trienoate (HHT) and malonaldehyde. The aqueous media half-life of PGH_2 has been reported as 5-30 min;[1] the modes of decomposition, with and without enzyme participation, are of obvious interest.



Scheme I

[1] The synthetic free acid (5) is reported to afford PGE2 together with traces of PGD2. The half-life in dry organic solvents is reported to be *ca* 3 hr at 25°C (7b).

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A number of simple model 2,3-dioxabicyclo[2.2.1]heptanes have been prepared (6-9), but their thermal, base catalyzed, and aqueous decomposition pathways do not mimic those reported for natural PGH_2. PGH_2 has also been synthesized (5), but unlike the more stable PGs (10,11) it has not been the subject of detailed structural investigation by high-field NMR methods,[2] nor has its chemistry been studied in detail. We now report the results of 500 MHz ^{1}H-NMR studies of: PGH_2, its Me ester (and some isosteric structural analogs), and the non-enzymic decomposition pathways of PGH_2 and its ester. These are intended as baseline studies for the use of ^{1}H-NMR as a tool for determining the mechanisms of enzymatic transformations involving PGH_2 and as a structure confirmation for thromboxane A_2 (TXA_2).[3]

Methods and Materials

$PGF_2\alpha$ Me ester, PGE_2, and PGD_2 used for these studies were products of previous synthetic efforts (14). 9α,11α- and 11α,9α-epoxymethano-15S-hydroxyprostadienoic acids were obtained as gifts from Dr. John Pike (Upjohn Co.). PGH_2 was prepared by the method of Green *et al* (15) utilizing a particulate fraction of FACO derived from frozen sheep seminal vesicles (SSV) (L.J. Marnett, Wayne State University). Typically the particulate fraction derived from 20 g of SSV in 80 ml of .1M KH_2PO_4 (containing 2mM phenol, 1mM EDTA, 1mM tryptophan, 0.1mg/ml hemoglobin, 1mM *p* hydroxymercuribenzoate) was treated with 5-8 mg of arachidonic acid (AA, Sigma). After 1 minute the incubation was quenched by additon of 2.5ml of 0.8M citric acid, and the cold ether extract was purified by silicic acid (.5 gm activated powder) column chromatography at 0-5°C. Elution with 6:4 ether:hexane afforded TLC homogeneous fractions of PGH_2 (55-40% yield) which were stored as a 1 mg/ml solution in acetone at -70°C. Ethereal CH_2N_2 treatment of PGH_2 afforded the Me ester which was similarly purified.

NMR Spectra were obtained for 1-10 mM solution of prostanoids in 99.5+% D C_6D_6, $(CD_3)_2CO$, and $CDCl_3$ or in 99.9+% phosphate buffered D_2O using a Bruker WM-500 FT-NMR spectrometer employing an Aspect 2000 computer for accumulation and Fourier transform by the standard programs.

High Resolution Spectra were obtained using the following parameters: 4-5 kHz spectral width, 10μs pulse, 32K channel (3.64s AQ) and 6s relaxation delay, 100-600 pulses. *Rapid Accumulation (low resolution)* spectra were 8K channel (AQ=1.0s, RD=0) and gave sufficient S/N at 1-2 mM level in 5-8 min (200 pulse cycles, ≈6 min). Under rapid accumulation conditions the integrals are not suitable for quantitation since resonances of differing T_1 show visably altered areas. *Transient* (selective inversion recovery) *NOE spectra* were accumulated as a direct difference FID_s by block interleaving using the pulse sequence, $[(RD\text{-}180^\circ_{on}\text{-}\tau\text{-}90^\circ FID)_8 NM(RD\text{-}180^\circ_{off}\text{-}\tau\text{-}90^\circ FID)_8]_{32}$, with RD=8.0 s, τ=400 ms, using a 40 ms decoupler pulse for the selective inversion. The 90° acquire pulse was 12.5 μs.

Results and Discussion

At 500 MHz the ^{1}H NMR of biosynthetic PGH_2 is remarkably detailed and a full assignment of all proton resonances (except H-17-19 which overlap severely)

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[2] The best previous ^{1}H-NMR of PGH_2 was recorded at 300 MHz for a $CDCl_3$ solution. In this spectrum only H-9,11,15, and 20 were specifically assigned (12).

[3] The structure announced in 1975 (15) has not been confirmed by any spectroscopic or analytic method due to the short half-life (37 s) of biosynthetic TXA_2 detected by bioassay or quenching techniques.

could be made *via* scalar decoupling experiments. Free acid PGH_2 displayed very large protodiastereomeric $\Delta\delta$ values for H-2,3 and 4; particularly in C_6D_6 solution. In addition, the C_6D_6 solution spectrum was highly concentration and temperature dependent. Similar, although less dramatic resolutions of protodia-

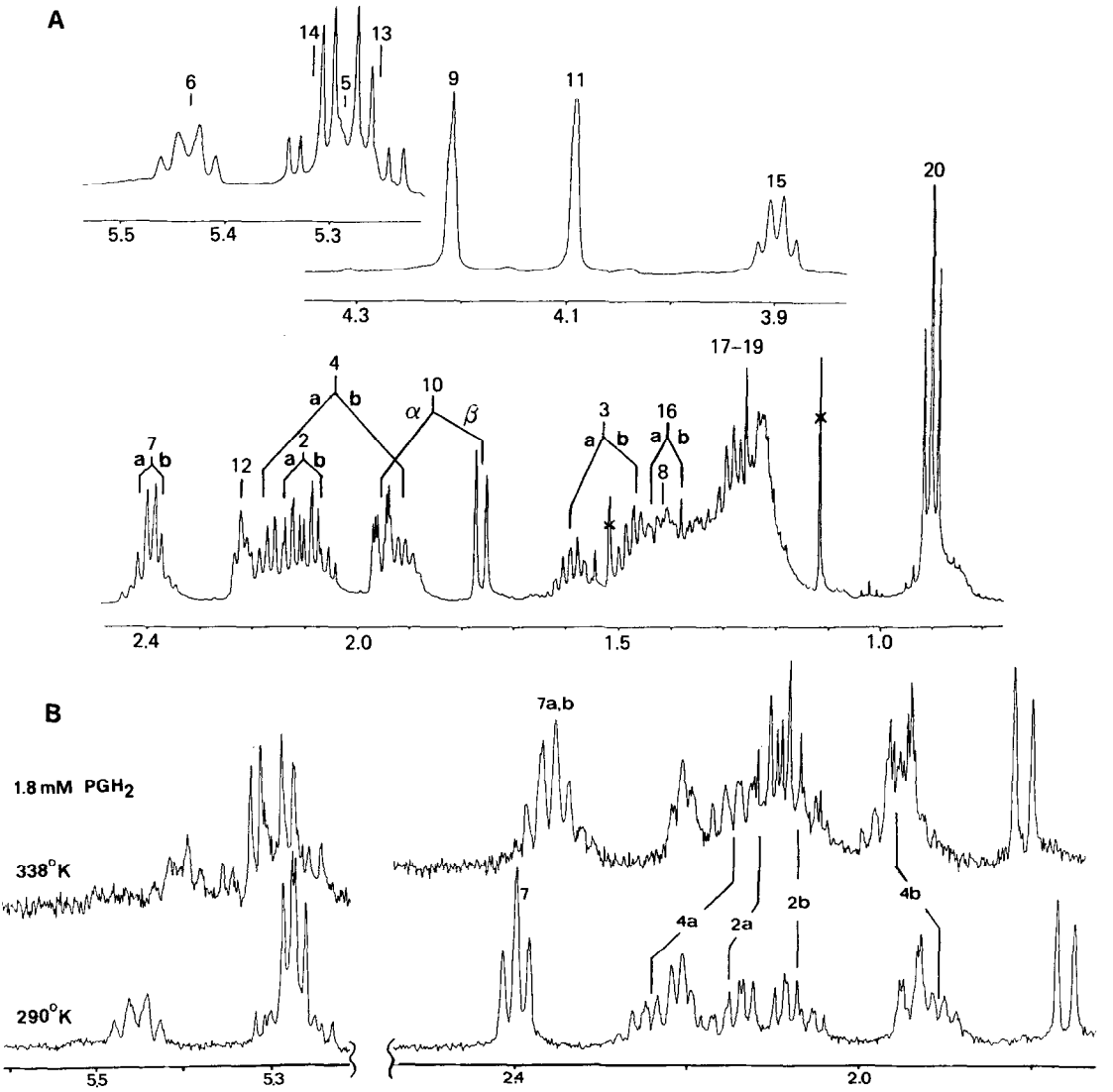
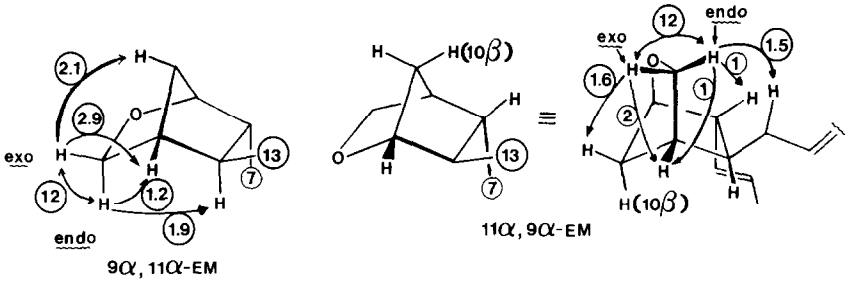


Figure 1. 500MHz spectra of PGH_2 in C_6D_6: upper panel (A), 10mM solution, 32K(RD=6s), 200 scans at 283°K, with assignments as derived from decoupling;[4] lower panel (B), 16K(RD=2s)-80 scan spectra of the same 1.8mM solution at 290 and 338°K. The particular high-temperature spectrum illustrated was accumulated in 5.5min at 1hr after the probe temperature had been raised to 338°K; no changes occurred over an additional hour at this temperature.

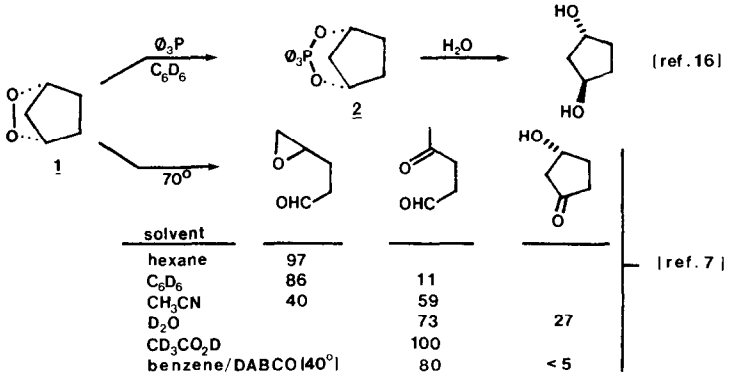
[4] H-15 could be assigned *a priori*. Irradiation at H-15 located H-14 (thus also H-13) and H-16a,b; H-13 irradiation revealed H-12; irradiation at H-12 served to locate H-8 and distinguish H-10α and β (only 10α has a significant coupling due to its planar zig-zig relationship); irradiation at 2.4 ppm (= H-7a,b) also decoupled H-8 and served to distinguish H-5 and 6; H-5 revealed H-4a,b; etc.



Nuclear Overhauser effects, as % fractional enhancements due to a 400 ms period of magnetization transfer (cross relaxation) with selectively inverted OCH_2 resonances. In this time period the OCH_2 *endo/exo* cross relaxation produces a mutual 12% enhancement.

stereomeric methylenes were observed for a variety of free acid prostanoids in non-polar media. We thus suggest that these remarkable chemical shift changes are due to carboxylate association rather than intrinsic conformational preferences in the α side-chain. Illustrative spectra appear in Figure 1.

The full spectral data for PGH_2 appears in Table I together with representative data for the epoxymethano-PGH_2 analogs (EMs) and $PGF_2\alpha$ derivatives. In 11,9- and 9,11-EM, one of the CH_2O resonances shows one substantial coupling (>2 Hz) other than the geminal coupling. This could most readily be ascribed to an *exo* bridgehead coupling in the bicycloheptane system. However, the inversion in relative chemical shift was surprising. We therefore performed selective inversion recovery difference NOE experiments for both CH_2O resonances in these two analogs. These served to confirm the bridgehead methine assignments and thus provide additional support for the 9,11 resonance assignments for PGH_2. The *exo* OCH_2 proton in each regioisomer shows significant cross-relaxation with the 10α proton (2-3%). As anticipated, the *endo*-OCH_2 proton of 9,11-EM shows substantial cross-relaxation with H-12α. The *endo*-CH_2O resonance of 11,9-EM was recognized from the 1.5% enhancement it induced at the C-7 methylene. A 1.0% enhancement of H-12, due to a 1,4 *endo/endo* interaction, also was observed. With the spectral assignments secured we turned to examining the reactions of PGH_2 by NMR in order to compare them to those reported for the unsubstituted model, Scheme II.



Scheme II

Table I. 500 MHz NMR Data for PGH_2, Its Isosteres and Degradation Products. **Bold face data** is for C_6D_6 solutions; data in other solvents are: *italic* (for D_2O), or gothic (for $CDCl_3$). Ring and side-chain numbering are as shown on the PGH_2 and TXA_2 structures in Scheme I.

	$PGF_2\alpha$ Me ester[f]	$PGF_2\alpha$ acid	PGH_2 acid[g,h]	9α,11α-EM[j]	11α,9α-EM[k]
H-2	**2.10; 2.322**	**2.20,2.15**; *2.16*	**2.134**[g],**2.076**; *2.25*[a]	2.34, 2.31	2.37[d],2.31
H-3	**1.59; 1.684**	**1.64,1.50**; *1.61*	**1.588,1.470**; *1.65*[b]	1.62, 1.55	1.759,1.67
H-4	**2.07,2.03; 2.11,2.09**	**2.24,2.07**; *2.05*	**2.177**[g],**1.912**[g]; *2.11*[a]	2.213,2.061	2.34[d],2.23
H-5,6	**5.33,5.47; 5.37,5.43**	**5.33,5.59**; *5.46,5.52*	**5.283,5.433**[g]; *5.45*[c],*5.48*[c]	5.476[c],5.34[c]	5.349,5.422
H-7	**2.35,2.17; 2.28,2.10**	**2.38,2.32**; *2.14*	**2.410,2.373**; *2.34*[a]	2.104,2.061	2.052,2.038
H-8	**1.44; 1.496**	*1.62*	**1.414**	1.60?	1.68
H-9,11	**3.91,3.79; 4.16,3.93**	**3.99,3.85**; *4.19,3.91*	**4.208,4.095**; *4.74,4.63*	4.174,2.300	2.460,4.030
H-10α,10β	**1.64,1.77; 1.75,2.20**	**1.68,1.83**; *1.52,2.48*	**1.952,1.763**[g]; -- ,*1.80*[b]	1.67 ±.015	1.706[e],1.622[e]
H-13,14	**5.38,5.50; 5.475,5.56**	**5.52,5.54**; *5.53,5.565*	**5.254,5.312**[g]; *5.507,5.53*	5.643,5.469	5.46 ±.01
H-12,15	**2.36,3.96; 2.33,4.05**	**2.32,4.06**; *2.25,4.13*	**2.230,3.897**; *2.29?,4.06*	1.819,4.116	2.003,4.102
H-16a,b	**1.53,1.43**	*1.60,1.50*	**1.44, 1.376**	1.56, 1.52	1.54, 1.48
Other	**3.34; 3.672 (OMe)**		**4.56,4.406(H-9,11)[$(CD_3)_2CO$]**	3.70(OCH_2, *exo*)[e]	3.56
				3.45(OCH_2, *endo*)[e]	3.83

[a,b,c,d] Resonance assignments that are tentative; all those with the same letter designation may be reversible.

[e] *Exo/endo* and 10α/β assignments follow from NOE data.

[f] Key coupling constants for C_6D_6 solution: 5.0(9,10β), 8.3 (11,10β), 6.3(14,15), 8.3(12,13), 3.5(11,10α), 1.1(9,10α).

[g] Many δ-values are temperature and concentration dependent in C_6D_6.

[h] The full set of coupling constants observed is: 16.2(2a,2b), 5.8(2a,3b), 6.3(2b,3a), 7.2(2a,3a;2b,3b), 13.8(3a,3b), 6.1(3a,4a), 7.7(3a,4b), 7.4(3b,4a), 5.1(3b,4b), 8.6 (4a,5), 7.7(4b,3a), 6.0(4b,5), 5.1(4b,3b), 10.6(5,6), 8.2(6,7a), 7.5(6,7b), 14.2(7a,7b), 8.0(7a,8), 5.5(7b,8), ≈2(8,9), ≈1(8,11), 4.6(8,12), ≈0.5(9,10β), 1.0(9,10α), ≈1(9,11), 10.5(10α,10β), 1.7(10α,11), 2.2(10α,12), <0.4(10β,11), 0.5(11,12), 6.7(12,13), 15.8 (13,14), 5.8(14,15), 5.8(15,16b), 7.4(15,16a), 7.2(19,20).

[j] The δ equivalence of 10α and β prevents the easy determination of many ring proton coupling values; some of the splittings observed are: ≈6.9(3,4), ≈1.5(8,9), 4.3(8,12), 6.8(OCH_2 *exo/endo*), 3.2(OCH_2 *exo*, 11β), ≈0.8(OCH_2 *exo*, 9β), ≈1(11,12), 7.1(12,13), 15.5(13,14), 6.8(14,15), ≈5.6(15;16a,b).

[k] 15.1(2a,b), 7.7(2,3), 7.4(3a,4b), 15.7(4a,4b), 7.7(4b,5), 6.6(4a,5), 1.3(4,6), 9.7(5,6), 1.1(5,7), 7.7(6,7), ≈4(8,12), ≈1.5(8,9), ≈0.6(9,OCH_2 *endo*), 2.4(9,OCH_2 *exo*), 0.4(OCH_2 *exo*, 11), 0.8(OCH_2 exo with 8 and 10β), 1.0(OCH_2 *endo*, 10β), 7.9(OCH_2 *exo/endo*), ≈2(10α,12), 10.9(10α,10β), 1.8(10α,11), ≈0.6(11,12), ≈5(12,13), 4.9(14,15), 6.2 and 6.6(15,16a and 15,16b), 13.0(16a,b).

Clennan and Heah (16) have recently reported exclusively *trans* cyclopentanediol from Ph_3P reduction of peroxide **1** and the detection of phosphorane **2** as a stable intermediate under anhydrous conditions. As shown on Scheme I, literature reports concerning PGH_2 indicate that $PGF_2\alpha$ is the only product. However, typical TLC systems do not distinguish between $PGF_2\alpha$ and 11-*epi*-$PGF_2\alpha$. Using boric acid coated SiO_2 TLC, we observe that Ph_3P reduction produces no (<4%) *trans* diols, 11-*epi*-$PGF_2\alpha$ or $PGF_2\beta$. The peroxy cleavage can also be monitored by NMR. No CHO-P methine signals at 4.4 - 4.9 ppm,

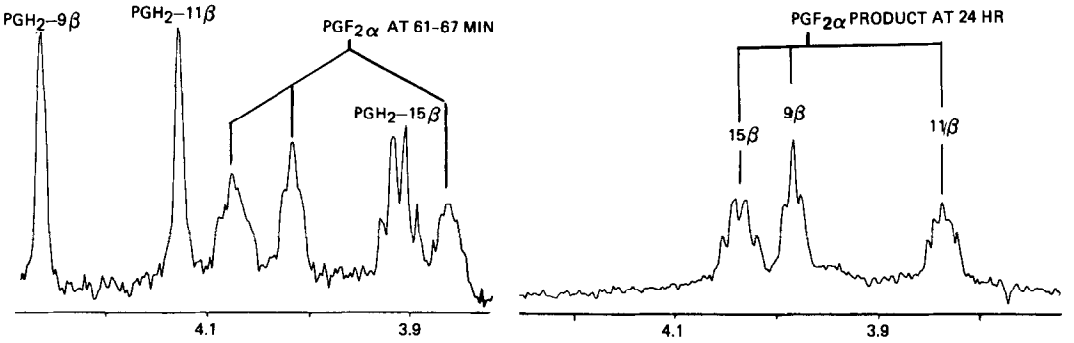
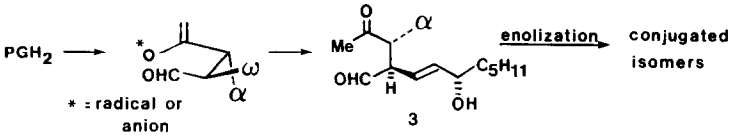


Figure 2. 500 MHz ^{1}H-NMR of 1.7mM PGH_2/C_6D_6 accumulated during and at the completion of reaction initiated by the addition of 2 equiv of Ph_3P. Spectra recorded in 8K channels (AQ=1s, RD=0, 200 pulses).

corresponding to phosphorane intermediates, were observed during the course of this reaction nor were $PGF_2\alpha$ diastereomers observed (Figure 2).[5]

These same methods can also be used to monitor the decomposition of PGH_2 in acid-buffered aqueous media (Figure 3). From this study we estimate that $t_{1/2}$ = 45min at 285°K, and that PGE_2 and PGD_2 are produced in a 3:1 ratio.[6] Endoperoxide model 1 is reported (7) to afford levulinaldehyde as the major product under comparable conditions. The comparable pathways for PGH_2 (speculatively illustrated in Scheme I), provide an alternative (but presumably non-stereospecific) route to PGE_2 and PGD_2. Both the NMR study and careful TLC work reveal that 11-*epi*-PGE_2 is not produced and thus the intermediacy of ketoaldelhydes appears unlikely.

In an attempt to prepare the fragmented ketoaldehydes from PGH_2, we monitored the spectrum of PGH_2 Me ester in C_6D_6 containing 20 mole-% DABCO at 298–307°C. Carboxaldehyde (δ 9.19, 9.56) and methyl ketone peaks (δ 2.08, 2.05) developed slowly over several days ($t_{1/2}$ ≈ 12hr @ 298°K); No PGE_2 could be detected. The δ values for the carboxaldehyde resonances suggest that the presumed initial product (3) undergoes further reactions to produce conjugated species (δ 9.4–9.8 in C_6D_6).



Natural PGH_2 has been reported to be thermally unstable ($t_{1/2}$≈3 hr, 298°K) while model 1 is more stable ($t_{1/2}$=2.9 hr, 346°K) (7b). Upon pyrolysis (GC detection) PGH_2 affords HHT and malonaldehyde, analogous products have not been

[5] This may simply reflect the difficulty in excluding stoichiometric water at these low concentrations.

[6] The longer half-life may be due to the lower pH (4–5) employed in our study. Ref. 4 reports 30 min at 20°C.

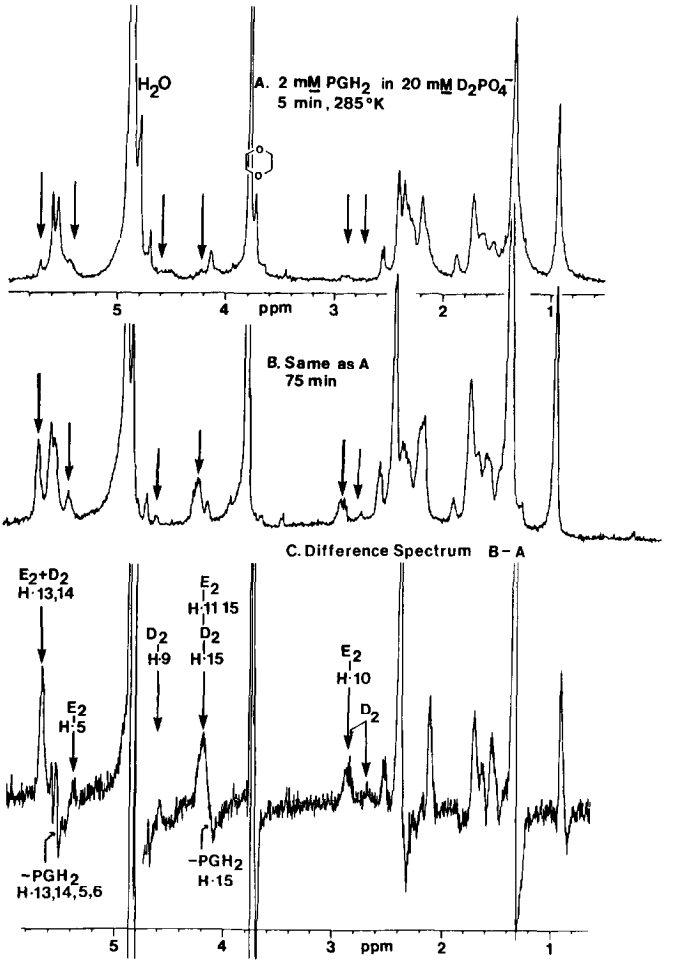


Figure 3. NMR Study of PGH_2 Decomposition in Aqueous Media. Not shown are reference spectra of PGE_2 and PGD_2, which were determined under the same conditions in order to ascertain the expectation δ-values (shown by arrows) in the decomposition spectra and to assign specific resonances.

observed for model 1; however the 1,4-diphenyl derivative does yield ethylene in low yield (≈10%) (6). Pure PGH_2 is much more stable than previous literature accounts suggest. As shown in Fig. 1 (panel B), no detectable conversion to HHT (or keto-PGs) is observed after 2 hrs at 338°K. The pure substance thus displays chemistry that stands in contrast to that seen in unsubstituted and symmetrically substituted models. The increased stability and changed modes of decomposition are most likely due to the torqueing of the dioxabicycloheptane skeleton caused by unsymmetrical substitution. More complete quantitative NOE studies should provide the details of the conformational changes that occur upon substitution. Such studies are in progress.

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