Prostaglandin Endoperoxide Reaction Mechanisms and the Discovery of Levuglandins

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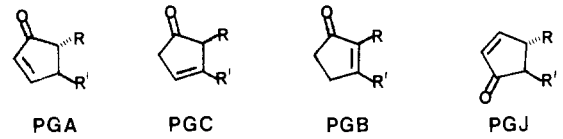
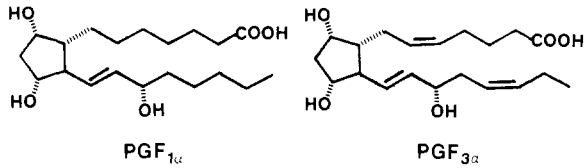
The complexity of biological systems often hampers elucidation of biosynthetic reactions and mechanisms. Isolation or even detection of molelcules which have biological importance can be thwarted by chemical instability, by high reactivity toward one or more of the huge variety of constituents in the biosynthetic mileu, by compartmentalization and heterogeneity of the biological environment, or by the minuscule concentrations which may be present in living systems. The oxidative conversion of polyunsaturated fatty acids into a vast array of potently biologically active natural products including prostaglandins and thromboxanes is typical. [Prostaglandin (PG) and thromboxane (TX) nomenclature refers to various ring structures, e.g., PGA, PGB, PGC, or PGJ with letters A through J (also





see Scheme I). The number of C=C bonds in the fatty acid side chains R and R′ are indicated by numerical subscripts, e.g., $PGF_{1\alpha}$ and $PGF_{3\alpha}$.] The first prostaglandin to be isolated, $PGF_{1\alpha}$, was originally detected in 1930 by bioassay, the biological response evoked by its presence.[1] But isolation and characterization were not accomplished until the early 1960s owing to the minute quantities available from natural sources.[2] That polyunsaturated fatty acids are biogenetic precursors of prostaglandins was demonstrated in 1964 by biosyntheses conducted outside living cells, in vitro bioconversion. Thus, by supplying the precursor fatty acids to crude tissue homogenates, prostaglandin biosynthesis was stimulated remarkably, and radiolabeled fatty acids yielded radiolabeled prostaglandins.[3] These discoveries had an immediate and profound impact on prostaglandin research. They provided an abundant supply of prostaglandins allowing extensive pharmacological testing. The powerful and diverse biological

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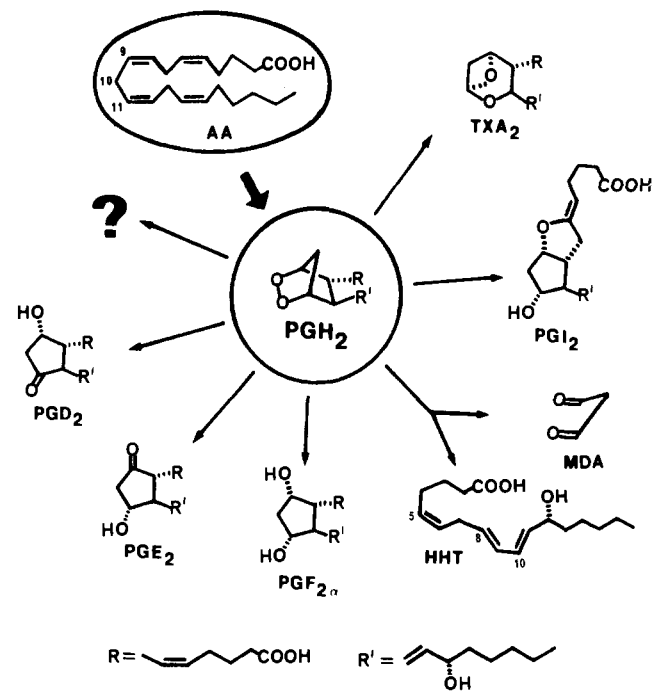
Scheme I



effects of prostaglandins on smooth muscle, respiratory, circulatory, reproductive, gastrointestinal, and neurological systems are now well documented.[4]

Several observations led to postulation of bicyclic peroxide intermediates, prostaglandin endoperoxides such as PGH_2, in prostaglandin biosynthesis (Scheme I).[5] Thus, bioconversion of 20-carbon fatty acids such as arachidonic acid (AA) into prostaglandins, i.e., PGD_2, PGE_2, and $PGF_{2\alpha}$, is accompanied by formation of polyunsaturated 17-carbon acids and a 3-carbon fragment, malondialdehyde (MDA).[5b] Isotopic labeling established that malondialdehyde is derived from carbon atoms 9–11 of the fatty acids. A plausible explanation for this side reaction seemed to be provided by fragmentation of 2,3-dioxabicyclo[2.2.1]heptane derivatives as in the production of 13-hydroxy-5,8,10-heptadecatrienoic acid (HHT) and malondialdehyde from PGH_2. Furthermore, isotopic labeling established that the two oxygen atoms on the cyclopentane ring of

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(4) For leading references see J. S. Bindra and R. Bindra "Prostaglandin Synthesis", Appendix 1, Academic Press, New York, 1977, pp 513–515.
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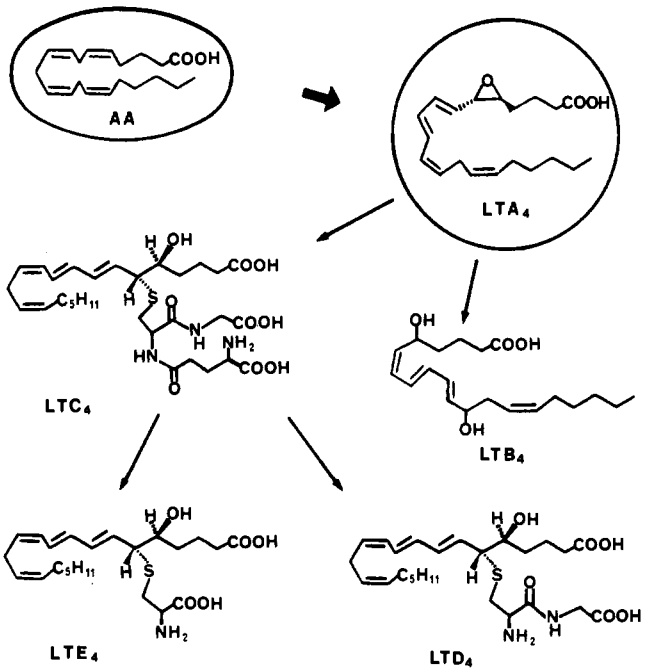
prostaglandins arise from a single molecule of oxygen.[6] Reductive cleavage of the O—O bond in a bridged bicyclic peroxide intermediate to produce F prostaglandins, and elimination across a C—O bond to generate the C=O bond in E and D prostaglandins could readily explain this result. Indeed, a bicyclic peroxide intermediate, PGH_2, was finally isolated in 1973.[7] The chemical instability of this peroxide ($t_{1/2}$ = 5 min at 37 °C) in the aqueous environment of its biosynthesis is noteworthy. Our efforts to obtain a fundamental understanding of this extraordinary reactivity and to explore prostaglandin endoperoxide chemistry through model studies began soon thereafter.

Concurrently, an even broader biosynthetic role for PGH_2 was demonstrated by a combination of bioassay and in vitro biosynthetic techniques. Earlier, aspirin was shown to be a potent inhibitor of prostaglandin biosynthesis from arachidonic acid.[8] However, the connection between this finding and the blood platelet antiaggregatory effects of aspirin was not obvious since PGE_2 and $PGF_{2\alpha}$ are only weakly proaggregatory.[9] An explanation for the strong antiaggregatory activity of aspirin was gleaned in 1975 when a platelet-bound enzyme was shown to generate a potent platelet proaggregatory agent, thromboxane A_2 (TXA_2), from PGH_2.[10] The structure of TXA_2 was not confirmed until 1985 when a total synthesis of this highly reactive molecule was finally achieved.[10b] The sensitive bioassay technique showed that TXA_2 is even less stable than PGH_2 owing to rapid hydrolysis in the aqueous environment of its biosynthesis ($t_{1/2}$ = 30 s at 37 °C). Aspirin's effect on TXA_2 biosynthesis is exerted through inhibition of the enzyme system "cyclooxygenase" which catalyzes the oxidative cyclization of arachidonic acid to PGH_2.[8] The intricacies of PGH_2 biochemistry are epitomized by the subsequent discovery of another branch in the cyclooxygenase pathway of arachidonic acid metabolism. Incredibly, enzymes embedded in arterial walls transform PGH_2 itself into a potently antiaggregatory agent, prostacyclin (PGI_2).[11] As for TXA_2, PGI_2 is rapidly inactivated by hydrolysis ($t_{1/2}$ = 2 min at 37 °C). Structural characterization of PGI_2 depended upon a comparison of the natural product with synthetic material prepared from $PGF_{2\alpha}$.[12]

Indeed, total synthesis can be virtually indispensable for some biochemical investigations. This is dramatically evident in the spectacular progress achieved since

Scheme II



1980 in elucidating a second route to biologically active products from arachidonic acid, the lipoxygenase pathway (Scheme II). This pathway involves an allylic epoxide intermediate, leukotriene (LT) A_4, which readily hydrolyzes ($t_{1/2}$ = 3 min) to the diol LTB_4. Total synthesis confirmed the structure of the reactive intermediate as well as those of its conjugates with various peptides (LTC_4 and LTD_4) or cysteine (LTE_4).[13] These conjugates, available in only minute quantities from natural sources, possess potent biological activities associated with the symptoms of asthma and other hypersensitivity reactions.[14]

The question mark in Scheme I symbolizes the fact that several observations suggested the formation of additional natural products derived from PGH_2 by yet another branch in the cyclooxygenase pathway (vide infra). Besides the large number of products derivable from PGH_2 and the relatively small quantities available even by in vitro bioconversion of arachidonic acid, the molecular complexity of PGH_2 and the derived products hampered direct investigation of its characteristic reactions and of their mechanisms. To surmount such obstacles we exploited studies of simplified model compounds to guide subsequent investigations of the less tractable natral products. The success of this protocol in unraveling the mysteries of prostaglandin endoperoxide reaction mechanisms and revealing additional products from PGH_2 will be detailed in the ensuing discussion.

Synthesis and Reactions of the Prostaglandin Endoperoxide Nucleus

Prior to the isolation of PGH_2, no derivatives of the 2,3-dioxabicyclo[2.2.1]heptane (1) ring system were known. Speculations[15] on the stability of PGH_2 were

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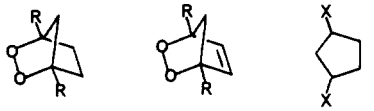
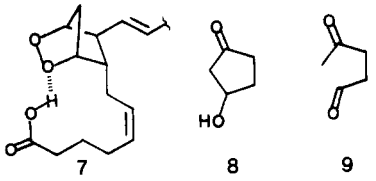


1, R = H 3, R = H 5, X = OSO_2CF_3
2, R = Ph 4, R = Ph 6, X = Br

based on analogy with peroxides such as **3** which decomposes explosively above −20 °C.[16] While PGH_2 proved considerably more stable than this, it does decompose readily, with a $t_{1/2}$ of only 5 min (37 °C), in the aqueous environment of its biosynethesis. A search for methods to prepare such peroxides was prompted by the availability of at most milligram quantities of biosynthetic PGH_2 and the possibility that the reported instability might be the result of catalytically active impurities.

Our discovery that diimide selectively saturates C–C π-bonds in the presence of the readily reducible O–O σ-bond allowed preparation of the first synthetic derivative of this ring system.[17] Diimide reduction of the cycloadduct **4** of 1,4-diphenyl-1,3-cyclopentadiene with singlet oxygen afforded **2**. This peroxide was unexpectedly stable toward thermal decomposition in benzene solutions showing an Arrhenius activation energy, E_a = 39 kcal/mol, identical with that for thermal decomposition of di-*tert*-butyl peroxide. Diimide reduction could also be exploited to produce the parent compound **1** from **3**.[18] However, the first syntheses of **1** were achieved in 1977 by alkylation of bis(tri-*n*-butyltin) peroxide with bis(triflate) **5** or alkylation of hydrogen peroxide with dibromide **6** in the presence of silver trifluoroacetate.[19] This synthetic method was also applied to the conversion of $PGF_{2\alpha}$ into PGH_2.[20]

The thermal stability of **1** in aprotic solvents is much greater than expected from observations on the decomposition of PGH_2. In benzene solution, decomposition of **1** occurs with a half-life of about 3 h at 70 °C. PGH_2 decomposes with a similar half life at only 20 °C in 1:1 petroleum ether–diethyl ether solution. In retrospect it is tempting to speculate that the relative instability of PGH_2 results from the effects of hydrogen bonding (vide infra) between the protic functional groups and peroxide moiety in PGH_2. Perhaps even intramolecular hydrogen bonding is involved as in **7**.



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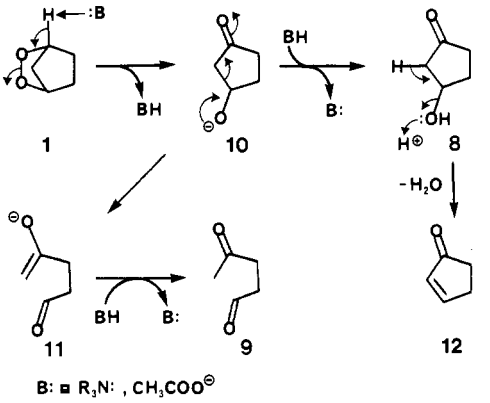
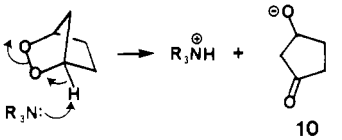
It seems likely that there is a basic center at the active sites of the enzymes which catalyze rearrangement of PGH_2 into PGE_2 and PGD_2. Rate-determining removal of a bridgehead proton from PGH_2 during these rearrangements is indicated by an isotope effect, k_H/k_D = 2–3, on the rate of formation of these products from

Scheme III



deuterated PGH_2 containing deuterium at the bridgehead position.[21] As a basis for anticipating the behavior of PGH_2 toward bases, we explored base catalyzed reactions of **1**. Immediately, our model studies revealed an unexpected transformation. Besides the anticipated elimination product **8**, tertiary-amine-catalyzed decomposition of **1** afforded a fragmentation product, levulinaldehyde (**9**).[19a] The basic center of the active site of enzymes which promote endoperoxide elimination is not necessarily an amino group. We also discovered that carboxylates, such as tetramethylammonium acetate, catalyze decomposition of **1**.[22] But again, both elimination and fragmentation reactions occurred. Even more intriguing was our observation that **9** is the major product from solvent-induced decomposition of **1** in aqueous solution.[23] Why had a similar rearrangement not been observed for PGH_2? In other respects **1** seemed to be an excellent model for PGH_2. One of the extraordinary reactions of PGH_2 is facile elimination to PGE_2 and PGD_2 in non-alkaline aqueous solution.[24] Not only does **1** undergo a similar rearrangement, yielding **8** but also the rates of decomposition of **1** and PGH_2 in aqueous solution are virtually identical. In fact fragmentation products *are* formed from PGH_2 (vide infra).

Amine-catalyzed elimination of dialkyl peroxides is known to involve rate-determining abstraction of an α-proton by the amine with concomitant cleavage of the peroxide bond.[25] For **1** this would generate a ketoalkoxide intermediate **10**. This suggests a mechanistic



hypothesis for the fragmentation of **1** to give **9** which accompanies base-catalyzed elimination to give hydroxy ketone **8**. As outlined in Scheme III, elimination and fragmentation reactions might share a common ketoalkoxide intermediate **10** which affords hydroxy ketone **8** by protonation and ketoaldehyde **9** by retroaldol fragmentation. This scenario leads to the expectation

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(21) P. Wlodawer and B. Samuelsson, *J. Biol. Chem.*, **248**, 5673 (1973).
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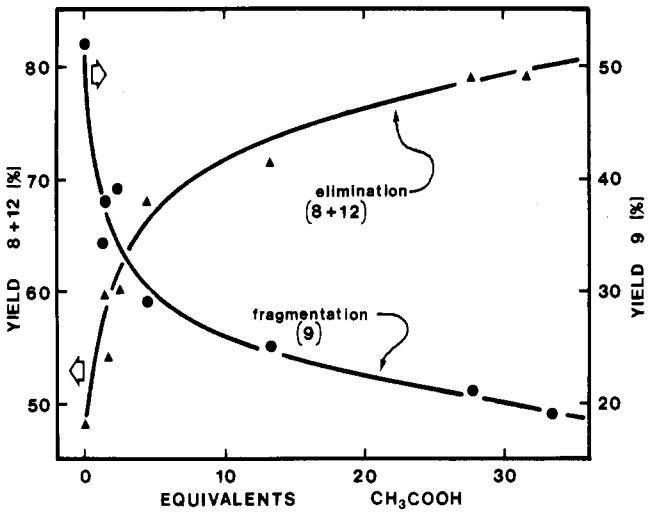
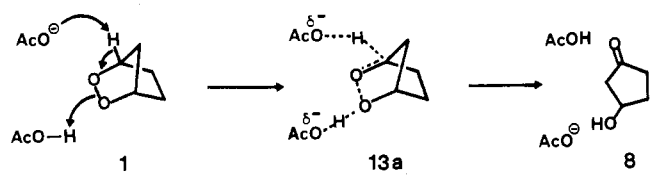


Figure 1. Correlation of elimination (▲) and fragmentation (●) yields with equivalents of acetic acid added to tetramethylammonium acetate (0.014 M) as catalyst.

that efficient protonation of **10** might favor elimination over fragmentation in the base-catalyzed decomposition of **1**.

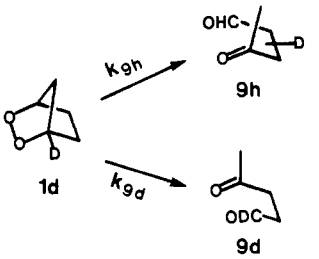
In fact, decomposition of **1** in the presence of both tetramethylammonium acetate as basic catalyst, and acetic acid as proton donor, also generated enone **12**.[22] This enone is formed by acid-catalyzed dehydration of **8**. Therefore, the combined yield of **8** and **12** equals the yield of elimination products. On the other hand, under the reaction conditions used, **8** does not undergo retroaldol cleavage to **9**, and **9** does not undergo aldol condensation to generate **8**. The effect of proton donor (i.e., acetic acid) concentration on the relative yields of fragmentation versus elimination products is dramatic (Figure 1).[22] Thus, fragmentation and elimination of **1** are equally important, 51 and 49%, respectively, with Me_4NOAc as catalyst. However, the relative yield of elimination increases sharply to 68% in the presence of about 4 equiv of HOAc and continues to rise to 79% with 0.46 M HOAc, 33 equiv relative to Me_4NOAc.

The influence of added acetic acid might be exerted after deprotonation of **1** by diverting a common ketoalkoxide intermediate **10** to hydroxy ketone **8** (Scheme III). Alternatively, the added acid might influence the product ratio by providing an additional route to **8** involving simultaneous bridgehead proton abstraction from **1** by acetate and protonation of the remote peroxidic oxygen by acetic acid as in **13a**. Since a ketoalkoxide is not generated in such a push–pull process, fragmentation could be circumvented.

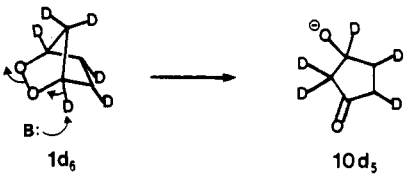


Evidence against the common ketoalkoxide intermediate hypothesis of Scheme III was secured by studies on the influence of deuterium substitution on the rates of base-catalyzed rearrangement of **1**. That bridgehead C–H bond cleavage occurs during the rate-determining step for *fragmentation* of **1** is evidenced by a huge difference in the rates of the **1** to **9** vs. $\mathbf{1d_6}$ to $\mathbf{9d_6}$ rearrangements. With the tertiary amine

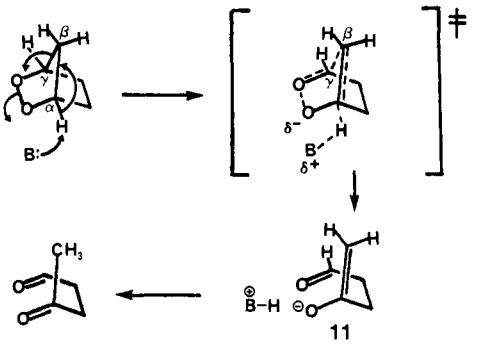
1,4-diazabicyclo[2.2.2]octane (DABCO) or the carboxylate Me_4NOAc as catalyst, an isotope effect $(k_H/k_D)_{9d6} = 7.9–8.3$ is observed for the rate of appearance of **9**.[26] However, a substantially smaller isotope effect is found for base-catalyzed fragmentation of **1d** which gives **9h** by cleavage of a bridgehead C–D bond and **9d** by cleavage of a bridgehead C–H bond. The isotope effect



$(k_H/k_D)_{9d} = k_{9d}/k_{9h} = 3.3–3.8$ with either DABCO or Me_4NOAc as catalyst. The great difference between $(k_H/k_D)_{9d}$ and $(k_H/k_D)_{9d6}$ reveals that cleavage of the bridgehead C–D bond is accompanied by a large secondary kinetic isotope effect owing to the other C–D bonds in $\mathbf{1d_6}$. Such large secondary isotope effects are not expected to accompany rate-determining generation of a ketoalkoxide intermediate $\mathbf{10d_5}$ from $\mathbf{1d_6}$. Instead,



these large secondary isotope effects suggest a novel rate-determining cleavage of three bonds, C_α–H, C_β–C_γ, and O–O during base-catalyzed fragmentation of **1**. This process generates the enolate **11** of levulinaldehyde



directly from the endoperoxide **1**. A change of the C_β–H and C_γ–H bonds from sp^3-s toward sp^2-s would accompany generation of the transition state. The consequent changes in the C–H bond force constants are expected to engender large secondary deuterium kinetic isotope effects if C–H is replaced by C–D.[27]

Most significantly, the influence of added acetic acid on the ratio of Me_4NOAc-catalyzed elimination vs.

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Table I
Solvent Effects for Decomposition of Endoperoxides 1 and 2,3-Dioxabicyclo[2.2.2]octane (14)

reaction solvent	dielectric constant[a]	relative rates: 1 at 73 °C	relative rates: 14 at 130 °C
cyclohexane-d_{12}	1.94	1.0[b]	1.0[c]
benzene-d_6	2.18	1.4	0.8
chlorobenzene	4.85	2.4	1.1
CD_3COOD	6.63	26.0	2.7
$ClCD_2CD_2Cl$	7.94	2.7	1.5
2-butanone	14.35	2.8	1.3
CD_3CN	28	4.4	1.8
D_2O	63	1.1×10^3[d]	6.2

[a] Estimated for 73 °C. [b] 4.4×10^{-5} s^{-1}. [c] 4.8×10^{-2} s^{-1}. [d] Extrapolated from rates measured between 21 and 46 °C.

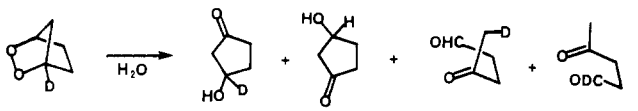
fragmentation of 1 supports the hypothesis that base-catalyzed transformation of a prostanoid endoperoxide can be channeled to hydroxycyclopentanone product (e.g., PGE_2 or PGD_2) by efficient protonation. This protonation apparently occurs, as shown in **13a** (vide supra), during rather than after a rate-determining cleavage of the bridgehead C–H bond. It is tempting to speculate that such bifunctionality might be found at the active site of enzymes which catalyze production of PGE or PGD from PGH. Although the requisite acidic and basic sites must be intimately juxtaposed, they could be held sufficiently apart that the endoperoxide substrate would not have to break a salt linkage, i.e., between a cationic acidic site and anionic basic site, in order to arrive at the catalyst–substrate complex. Furthermore, these sites could be rigidly positioned to optimize steric effects[28] and the spatial arrangement of reacting centers, i.e., "approximation".[29]

The extraordinary instability of PGH_2 and the model peroxide 1 in aqueous solution is a characteristic of the 2,3-dioxabicyclo[2.2.1]heptane ring system. The data presented in Table I[26] revealed two important facts. Protic solvents exert a profound destabilizing influence on the prostaglandin endoperoxide nucleus 1 compared with the aprotic solvents of similar dielectric constant, and a similar effect is *not* observed for the closely homologous peroxide 2,3-dioxabicyclo[2.2.2]octane (14).

14

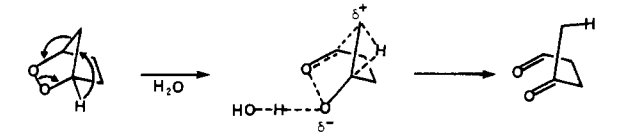
Thus, the rate of decomposition of 1 increases slightly with solvent polarity as measured by dielectric constant. However, the rate of decomposition in acetic acid is an order of magnitude higher, and the rate of decomposition in aqueous solution is at least 2 orders of magnitude greater than expected for solvents of similar dielectric constant. This contrasts with the decomposition of 14 for which the rate in acetic acid or aqueous solution is not substantially greater than expected for solvents of similar dielectric constant.

Water-induced decomposition of $\mathbf{1d_6}$ is four times slower than decomposition of 1. This large deuterium kinetic isotope effect reveals that rate-determining cleavage of the bridgehead C_α–H bond occurs during rearrangement of 1 to 8 or 9 *or* both products. That water-induced rearrangements of 1 to 8 and 9 *both* involve rate-determining cleavage of a bridgehead C_α–H bond was demonstrated by examining deuterium distributions in the products from decomposition of **1d**.



1d 3-d-8 8 5-d-9 1-d-9

The ratio of C_α–H vs. C_α–D cleavage during fragmentation of **1d** is given by 5-d-9/1-d-9 = 1.5. In contrast, the ratio of C_α–H vs. C_α–D cleavage during elimination is given by 3-d-8/8 = 3.3. Thus, the rate-determining steps of the solvent-induced fragmentation and elimination reactions of endoperoxide 1 in aqueous solution are very different although both involve cleavage of a bridgehead C–H bond. A common intermediate is not involved in the formation of the fragmentation product 9 and elimination product 8. Significantly, no deuterium is lost during fragmentation of **1d** although a C_α–D bond is cleaved during formation of 5-d-9. If the H_2O solvent were serving as a base, abstracting D^+ from **1d**, the product $[H_2OD]^+$ would certainly sometimes transfer a proton to the resulting levulinaldehyde enolate 11. Evidently, water-induced fragmentation of 1 involves a novel intramolecular 1,2-hydride shift accompanying concerted cleavage of three bonds. The transition state in such a mechanism could be stabilized by a hydrogen-bonding interaction with the solvent as depicted in **13w**.



1 13w 9

Discovery of Levuglandins

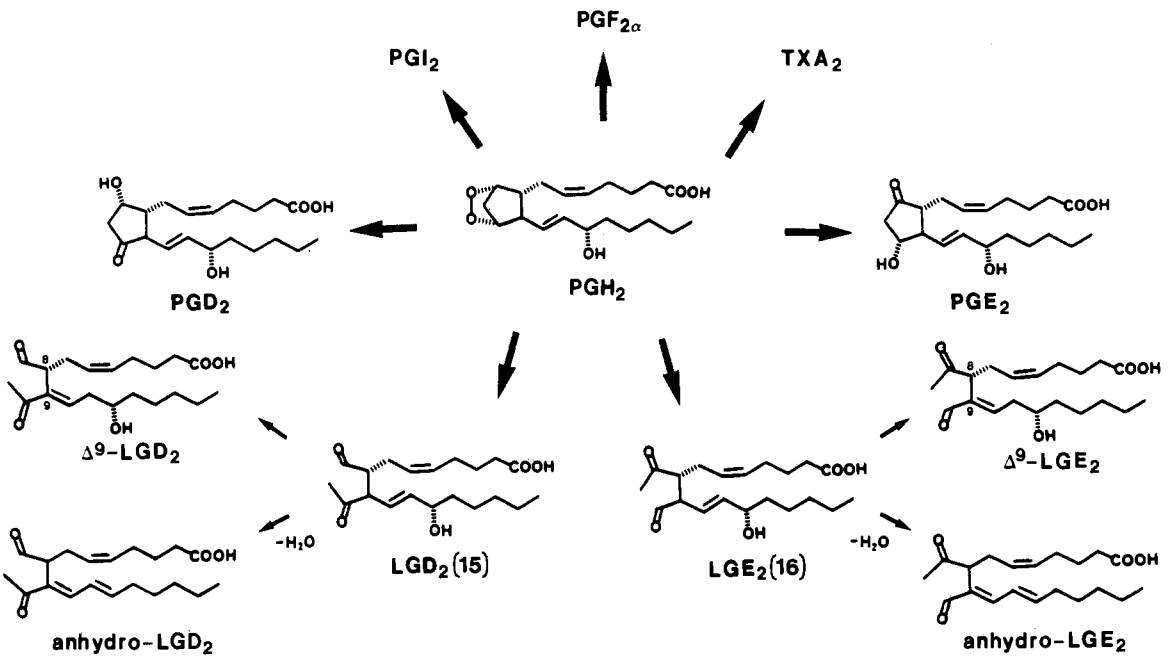
In view of our model studies, we anticipated that PGH_2 must produce levulinaldehyde derivatives by fragmentations analogous to the 1 to 9 rearrangement. Indeed, decomposition of PGH_2 in pH 7.9 *phosphate*-buffered aqueous solution produces two aldehydes in 22% combined yield together with prostaglandins.[22] The new aldehyde products 15 and 16 from PGH_2 were named levuglandin (LG) D_2 and LGE_2, respectively, since these derivatives of levulinaldehyde are *formally* related to PGD_2 and PGE_2 by aldol condensation. Both of these new primary products from PGH_2 are chemically sensitive vinylogous β-hydroxycarbonyl compounds which loose water to produce anhydrolevuglandins and undergo allylic rearrangement to produce Δ^9 isomers (Scheme IV). In pH 7.8 0.01 M phosphate-buffered aqueous solution LGE_2 affords anhydro-LGE_2 with $t_{1/2}$ = 12 h at 25 °C. Interestingly, decomposition is slightly faster in 0.1 M phosphate buffer and besides anhydro-LGE_2, an equal amount of α,β-unsaturated isomer Δ^9-LGE_2 is formed.[30] Isolation of pure LGD_2 remains a problem since this levuglandin dehydrates even more readily than LGE_2. ^1H NMR and mass spectral data[31] were deemed inadequate for un-

(28) (a) C. J. Collins and N. S. Bowman, Eds. "Isotope Effects in Chemical Reactions", Van Nostrand-Reinhold, New York, 1970. (b) V. J. Shiner, Jr. in "Isotopes and Chemical Principles", P. A. Rock, Ed., American Chemical Society, Washington, D.C., 1975, *ACS Symp. Ser. No. 11.*

(29) A. Streitwiser, Jr. "Solvolytic Displacement Reactions", McGraw Hill, New York, 1962, p 172.

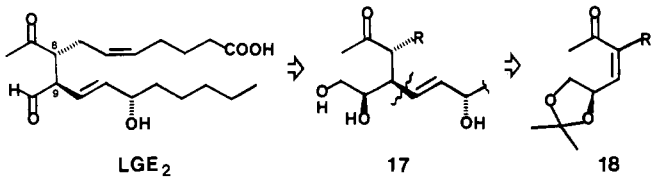
(30) D. B. Miller, unpublished results.

Scheme IV

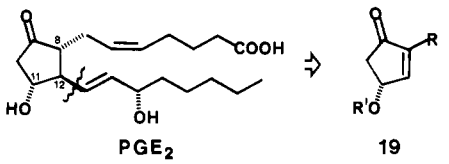


ambiguous distinction between E_2 and D_2 levuglandins. The structures were firmly established by total syntheses of anhydro-LGD_2[32] and LGE_2.[33]

A short, convergent, and remarkably stereoselective synthesis of LGE_2 was achieved by 1,4-addition of a vinyl nucleophile to an enone 18.[33] Stereoselective

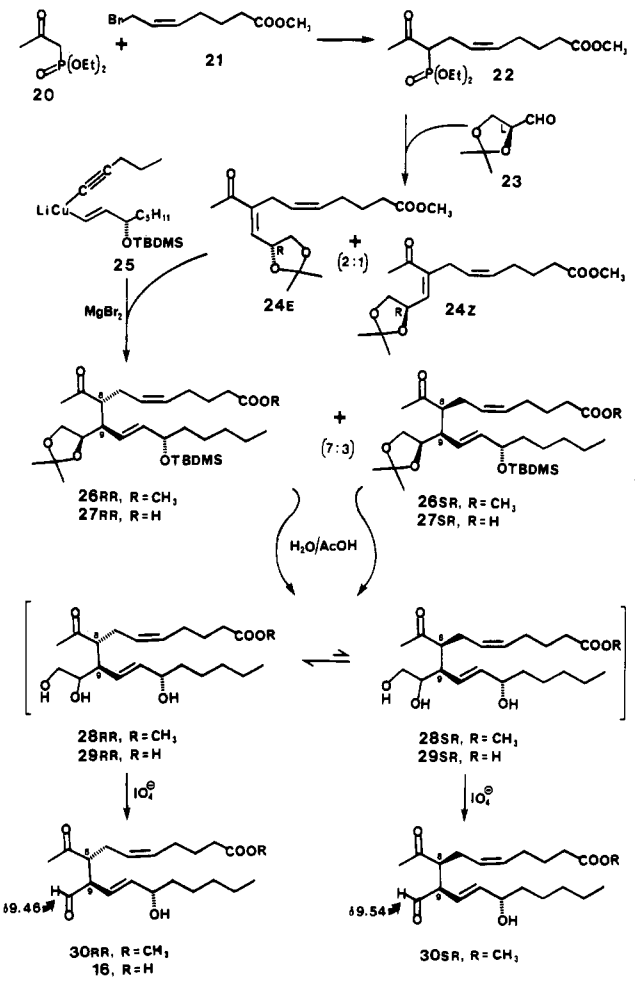


synthesis of E prostaglandins is readily achieved by 1,4-addition of vinyl nucleophiles to cyclopentenones **19** bearing a single asymmetric center at the incipient 11-position.[34] Steric approach control engenders a



trans relationship between the substituents at positions 11 and 12. While E prostaglandins can readily epimerize at position 8 through enolization of the vicinal carbonyl group, a strong thermodynamic preference assures the correct trans relationship between substituents at positions 8 and 12. The acylic levuglandins present a more challenging target for stereocontrolled total synthesis. Recent precedent indicated that an *R* configuration at the alkoxy-substituted allylic center in 18 would foster generation of the required *R* configuration at position 9 in LGE_2 during 1,4-addition of a vinyl nucleophile.[35] However, it was not obvious that

Scheme V

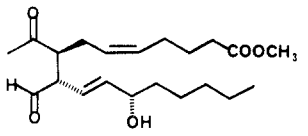


equilibration of epimers at position 8 would be the key to favorable stereoselection at this center. An important feature of our synthetic strategy is generation of the sensitive vinylogous β-hydroxyaldehyde functional array late in the synthesis employing a mild cleavage of a

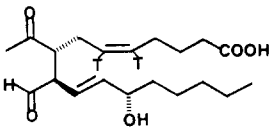
(31) R. G. Salomon and D. B. Miller, *Adv. Prostaglandin, Thromboxane, Leucotriene Res.*, **15**, 323 (1985).
(32) B. S. Levison, D. B. Miller, and R. G. Salomon, *Tetrahedron Lett.*, **25**, 4633 (1984).
(33) R. G. Salomon, D. B. Miller, S. R. Raychaudhuri, K. Avasthi, K. Lal, and B. S. Levison, *J. Am. Chem. Soc.*, **106**, 8296 (1984).
(34) C. J. Sih, R. G. Salomon, P. Price, R. Sood, and G. Peruzzotti, *J. Am. Chem. Soc.*, **97**, 857 (1975) and references cited therein.
(35) W. R. Roush and B. M. Lesur, *Tetrahedron Lett.*, **24**, 2231 (1983).

vicinal diol **17** with periodate.[36] The LGE_2 skeleton was assembled as outlined in Scheme V. Alkylation of (diethylphosphono)acetone (**20**) with methyl 7-bromohept-5(*Z*)-enoate[37] (**21**) affords **22** whose sodium salt reacts with isopropylidene-L-glyceraldehyde (**23**) to produce the isomeric enones **24**-*Z* and **24**-*E* (1:2.3). Reaction of either isomeric enone with vinyl cuprate **25**[38] prepared from 1-iodo-3(*S*)-[(*tert*-butyldimethylsilyl)oxy]-1(*E*)-octene[39] afforded identical mixtures of **26**-*RR*[40] (70%), which has the C-8 configuration of LGE_2, and the C-8 epimer **26**-*SR*[40] (30%). Interestingly, Michael addition occurred only in the presence of $MgBr_2$, which presumably serves as a Lewis acid catalyst.[41] That **26**-*RR* and **26**-*SR* are epimeric at position 8 is evident from the observation that saponification of either ester afforded identical mixtures of epimeric acids **27**-*RR* (70%) and **27**-*SR* (30%). The selective generation of an *R* configuration at position 9 in **26** provides yet another example of the remarkably high acylic stereoselection achievable in 1,4-addition reactions.[35,42]

Unexpectedly, treatment of either **26**-*RR* or **26**-*SR* with aqueous acetic acid *followed by sodium periodate* afforded the same 13:1 mixture of LGE_2 methyl ester (**30**-*RR*) and the 8-epi isomer **30**-*SR*. This remarkably high preference for the required 8*R* configuration contrasts with the 7:3 equilibrium ratio observed for **27**-*RR* and **27**-*SR*. The 1,2-dihydroxyethyl substituent in the intermediate vicinal diol **28**-*SR* apparently plays a role in the *stereosective epimerization to* **28**-*RR* since efficient interception of this intermediate allows conversion of **26**-*SR* to mixtures richer in the **30**-*SR* epimer. Thus, treatment of **26**-*SR* with aqueous acetic acid *in the presence of sodium periodate* yielded a 2:1 mixture of **30**-*RR* and **30**-*SR*. For comparison with LGE_2 obtained by solvent-induced rearrangement of PGH_2, 8-*epi*, 9-*epi*-LGE_2 methyl ester (**30**-*SS*) was prepared



30SS

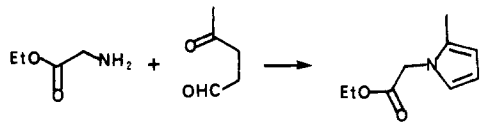


t_2-16

by substituting isopropylidene-D-glyceraldehyde for the L isomer **23** in Scheme V. Comparison of LGE_2 methyl ester derived from PGH_2 with the synthetic epimers of **30** showed that epimerization of LGE_2 at positions 8 or 9 is not extensive under the conditions of its solvent-induced generation from PGH_2.[33] Levuglandin E_2 (**16**) was prepared in good yield from the ketal **29**-*RR* (or **29**-*SR*) by treatment with aqueous acetic acid followed by sodium periodate. This efficient synthesis provides an abundant source of LGE_2 and its unnatural epimers for studies of their chemical reactions and biological activities. The tritiated derivative t_2-**16**, prepared by a minor modification of Scheme V, is now available for studies on covalent binding of levuglandins with biological nucleophiles and may eventually permit detection of levuglandins in biological systems by the sensitive radioimmunoassay technique.[43]

It seems probable that levuglandins are formed in vivo since we have now established that solvent-induced nonenzymatic generation of prostaglandins is accompanied by formation of significant quantities of levuglandins. Solvent induced conversion of PGH_2 competes effectively with the corresponding enzyme mediated conversion under a variety of conditions.[44] It still remains to be determined whether levuglandins are also produced in enzymic or other catalyzed transformations of PGH_2, e.g., decomposition catalyzed by human serum albumin.[45]

Previous model studies showed that levulinaldehyde (**9**) forms covalent adducts with primary amines. The pyrrole **31** was isolated from the reaction of glycine ethyl ester with **9**.[26] As anticipated, therefore, we found



9 31

that LGE_2 reacts with tris(hydroxymethyl)aminomethane (TRIS).[31] This probably explains the apparent conflict of our results with those of several previous investigations which failed to detect levuglandins from the decomposition of [1-^{14}C]-PGH_2 in TRIS-buffered aqueous solution.[24,45a,46] In one study, products isolated by extraction, separated and identified by TLC, and quantified by liquid scintillation counting included PGE_2 (71%) and PGD_2 (20%) and no levulinaldehyde derivatives.[24] However, it is important to recognize that these are distributions of products isolated and not absolute yields. Perhaps, covalent adducts formed between levuglandins and the TRIS buffer were not isolated.

The presence of several reactive electrophilic centers in levuglandins may also complicate their detection in living systems. Besides the electrophilic carbonyl groups, the α,β-unsaturated Δ^9-levuglandins and anhydrolevuglandins are Michael acceptors. Covalent adduct formation might occur with biological nucleophiles such as thiol or primary amino groups, e.g., of

(36) An early application of a vicinal diol as a latent carbonyl appears in a synthesis of lysergic acid: E. C. Kornfeld, E. J. Fornefeld, G. B. Klein, M. J. Mann, D. E. Morrison, R. G. Jones, and R. B. Woodward, *J. Am. Chem. Soc.*, **78**, 3087 (1956).

(37) (a) J. Martel and E. Toromanoff, German patent 2 121 362, 1971; *Chem. Abstr.*, **76**, 24712 (1972). (b) E. J. Corey and H. S. Sachdev, *J. Am. Chem. Soc.*, **95**, 8483 (1973).

(38) E. J. Corey and D. J. Beames, *J. Am. Chem. Soc.*, **94**, 7210 (1972).

(39) A. G. Kluge, K. G. Untch, and J. H. Fried, *J. Am. Chem. Soc.*, **94**, 7827 (1972).

(40) For compounds **26**–**30** the designations *RR*, *SR*, and *SS* refer to the absolute configurations of the chiral centers at C-8 and C-9, respectively.

(41) Y. Yamamoto, S. Yamamoto, H. Yatagai, Y. Ishihara, and K. Maruyama, *J. Org. Chem.*, **47**, 119 (1982) and references cited therein.

(42) (a) K. C. Nicolaou, M. R. Pavia, and S. P. Seitz, *Tetrahedron Lett.*, 2327 (1979); *J. Am. Chem. Soc.*, **103**, 1224 (1981); **104**, 2027 (1982); (b) M. Isobe, M. Kitamura, and T. Goto, *Tetrahedron Lett.*, 3465 (1979); **21**, 4727 (1980); (c) K. Tatsuta, Y. Amemiya, S. Maniwa, and M. Kinoshita, *ibid.*, **21**, 2840 (2980); (d) K. Tatsuta, Y. Amemiya, Y. Kanemura, and M. Kinoshita, *ibid.*, **22**, 3997 (1981); (e) F. E. Ziegler and P. J. Gilligan, *J. Org. Chem.*, **46**, 3874 (1981); (f) C. Fuganti, P. Graselli, and G. Pedrocchi-Fantoni, *Tetrahedron Lett.*, **22**, 4017 (1981).

(43) S. Ghosh, D. B. Miller, R. B. Sharma, and R. G. Salomon, unpublished results.

(44) (a) D. B. McNamara, J. L. Hussey, M. D. Kerstein, R. S. Rosenson, A. L. Hyman, and P. J. Kadowitz, *Biochem. Biophys. Res. Commun.*, 118, 33 (1984); (b) F. F. Sun, J. P. Chapman, and J. C. McGuire, *Prostaglandins*, **14**, 1055 (1977); (c) F. F. Sun, *Biochem. Biophys. Res. Commun.*, **74**, 1432 (1977); (d) A. J. Marcus, B. B. Weksler, E. A. Jaffe, L. Safier, H. Ullman, M. Brockman, C. Dorso, and S. Brown, *Prog. Lipid Res.*, **20**, 431 (1981).

(45) (a) D. H. Nugteren and E. Christ-Hazelhof, *Adv. Prostaglandin Thromboxane Res.*, **6**, 129 (1980); (b) M. Hamberg and B. B. Fredholm, *Biochim. Biophys. Acta*, **431**, 189 (1976).

(46) C. J. Sih, C. Takeguchi, and P. Foss, *J. Am. Chem. Soc.*, **92**, 6670 (2970).

various peptides, proteins, or nucleic acids. Indeed, levuglandins may account for previous observations of covalent adduct formation between unidentified products of the cyclooxygenase pathway and microsomal proteins or glutathione[47] and human serum albumin[48] as well as DNA.[49] Two interesting consequences of this binding were noted. Thus SDS polyacrylamide gel electrophoresis showed protein polymerization suggesting that covalent binding results in cross-linking. Also, covalent binding with protein or DNA is accompanied by the appearance of a fluorescent pigment. Pigment formation and cross-linking were also associated with the neuropathic binding of γ-diketones[50] such as 3,4-dimethyl-2,5-hexanedione which are structural analogues of the γ-ketoaldehyde levuglandins. It is interesting in this connection that botryodiplodin, an antibiotic levulinaldehyde derivative, causes pigment formation, i.e., "pink stains", when applied to human skin.[51] Thus, levuglandins may be involved in the pigment (lipofuscin) formation which is associated with ageing and heart damage.[52]

(47) (a) M. W. Anderson, D. J. Crutchley, A. Chaudhari, A. G. E. Wilson, and T. E. Eling, *Biochim. Biophys. Acta*, 40 (1979); (b) D. J. Crutchley, H. J. Hawkins, T. E. Eling, and A. W. Anderson, *Biochem. Pharmacol.*, **28**, 1519 (1979); (c) T. E. Eling, A. G. E. Wilson, A. Chaudhari, and M. W. Anderson, *Life Sci.*, **21**, 245 (1977).

(48) J. Maclouf, H. Kindahl, E. Granstrom, and B. Samuelsson, *Adv. Prostaglandin Thromboxane Res.*, **6**, 283 (1980).

(49) S. Vasdav and P. J. O'Brien, *Prostaglandins Relat. Lipids*, **2**, 163 (1982).

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(52) J. Miguel, J. Oro, K. G. Bensch, and J. E. Johnson, Jr., in "Free Radicals in Biology", Vol. 3, W. A. Pryor, ed., Academic Press, New York, 1977, pp 133–82.

Preliminary results demonstrate that levuglandins are biologically active. Both Δ^9-LGE_2 and 8-epi-Δ^9-LGE_2 as well as anhydro-LGE_2 and anhydro-LGD_2 induce strong contractions in rat uterus at concentrations less than 200 ng/mL.[53] This tissue is nearly an order of magnitude more sensitive to $PGF_{2\alpha}$ under the same bioassay conditions.

Conclusions

Occasionally Nature provides us with molecules that not only have unusual structures but also exhibit extraordinary chemical reactivity which is crucial to their biological roles. PGH_2 is a pertinent example. Our studies established that the chemistry of 2,3-dioxabicyclo[2.2.1]heptane, the reactive bicyclic nucleus of PGH_2, is unique and cannot be predicted by simple extrapolation of the behavior of any other dialkyl peroxide. The power of studies on simplified models to reveal important details, not readily discerned from studies on the natural products themselves, is demonstrated by our seminal discovery that levuglandins are major products of the solvent-induced rearrangement of PGH_2 under the aqueous conditions of its biosynthesis.

For their industrious, meticulous, persistent, and thoughtful collaboration in the research described herein, I wish to thank my students whose names appear in the references. For financial support of this research, my students and I thank the Division of General Medical Sciences of the National Institutes of Helath (Grant GM-21249), G. D. Searle and Co., the Research Corp. and the Northeast Ohio Chapter of the American Heart Association (Grant 4313).

(53) D. Foreman, L. Zuk, D. B. Miller, and R. G. Salomon, unpublished results.