product is not thiocyanate but either the product of its further reaction with BrCN according to eq 1 or a further decomposition product of $S(CN)_2$.

$$BrCN + {}^{-}SCN \rightarrow Br^{-} + S(CN)_2 \quad (1)$$

The reactions of ADPαS and ATPβS are not useful for synthesizing adenosine 5′-[^{18}O]diphosphates or -triphosphates with chiral P_α or P_β because of the oxygen rearrangements in the polyphosphate systems described herein. These can be prevented by protecting the terminal phosphoryl groups with removable alkyl substituents, as demonstrated by the reactions of the R_P and S_P epimers of β-(cyanoethyl)-ADPαS with BrCN in $H_2{}^{18}O$ to produce high yields of the S_P and R_P epimers of β-(cyanoethyl)-[α-^{18}O]ADP.[22] The displacement of thiocyanate by $H_2{}^{18}O$ proceeded with inversion of configuration at P_α, and the β-cyanoethyl groups were easily removed by treatment with base to produce the S_P and R_P epimers of [α-^{18}O]ADP.[22]

Eckstein and Lowe and their collaborators have been able to desulfurize nucleoside phosphorothioates with electrophilic brominating agents in acidic solutions with inversion of configuration and without rearrangements in polyphosphates.[19c,23] At neutral pHs these reactions also involved rearrangements, suggesting that our observations do not represent an isolated phenomenon observable only in the special case of reactions of BrCN with ADPαS or ATPβS.

This paper and our earlier communication[4] provide the first evidence for involvement of *cyclo*-diphosphates in chemical reactions. Dimeric phenylphosphonic anhydride is the only four-membered ring organophosphorus compound reported in the literature that has two P–O–P bonds.[24,25] Recently a cyclic phosphoric acid anhydride was postulated as a possible intermediate in the equilibration of the phosphorus in $H_3{}^{32}PO_4$ with that of $POCl_3$.[26]

cyclo-Dephosphates have never been proposed as intermediates in enzymatic reactions. In view of their involvement in nonenzymatic reactions proceeding to completion within a few minutes in aqueous solutions at physiological pHs and moderate temperatures, they should be considered as possible intermediates in enzymatic reactions. However, the substrates for most nucleoside di- and triphosphate-dependent enzymes are the metal complexes of the nucleotides rather than the nucleotides themselves, and it is not known what effect metal complexation would have on the rearrangements described here. It is possible that these rearrangements would be inhibited or prevented by metal chelation. Moreover, there is no reason to consider invoking *cyclo*-diphosphates as intermediates in ordinary phosphotransferase or nucleotidyl transferase reactions. Nevertheless, the ease and speed with which *cyclo*-diphosphates appear and react under mild conditions demonstrate their potential as transient intermediates that might be involved in more complex biological reactions.

Acknowledgment. This research was supported by Grant GM30480 from the National Institute of General Medical Sciences. Purchase and operation of the Bruker WP-200 NMR spectrometer was supported by Grant GM27431. Spectra were also obtained at The Ohio State University Chemical Instrument Center using a Bruker WM-300 spectrometer funded in part by NSF Grant CHE 7910019. We are grateful to Mel Micke for his assistance in obtaining mass spectral data.

Registry No. 4, 91711-81-8; **5**, 91741-70-7; **10**, 91741-71-8; cyanogen bromide, 506-68-3; (S_P)-[β-^{18}O]ATPBS, 87226-46-8; (S_P)-[β,γ-bridging-^{18}O]ATPBS, 87883-26-9; (R_P)-[β-^{18}O]ADPBS, 69182-10-1; (S_P)-[β-^{18}O]ADPBS, 68973-41-1; (S_P)-ATPBS, 59261-36-8; (S_P)-ADPαS, 59286-20-3; (S_P)-[α-$^{18}O_2$]ADPαS, 91711-80-7; [α-^{18}O,β-^{18}O]ADP, 91711-82-9; [β-^{18}O,γ-^{18}O]ATP, 91711-83-0.

(22) Sammons, R. D.; Frey, P. A. *J. Biol. Chem.* **1982**, *257*, 1138–1141.
(23) Connolly, B. A.; Eckstein, F.; Fülder, H. H. *J. Biol. Chem.* **1982**, *257*, 3382–3384.
(24) Michaelis, A.; Rotte, F. *Ber. Dtsch. Chem. Ges.* **1892**, *25*, 1747–1752.
(25) Fluck, V. E.; Binder, H. *Z. Anorg. Alleg. Chem.* **1967**, *354*, 139–148.
(26) Keenan, R. W.; Martinez, R. A.; Williams, R. F. *J. Biol. Chem.* **1982**, *257*, 14817–14820.

Solvent-Induced Fragmentation of Prostaglandin Endoperoxides. New Aldehyde Products from PGH_2 and a Novel Intramolecular 1,2-Hydride Shift during Endoperoxide Fragmentation in Aqueous Solution[1]

Robert G. Salomon,* Donald B. Miller, Michael G. Zagorski, and Daniel J. Coughlin

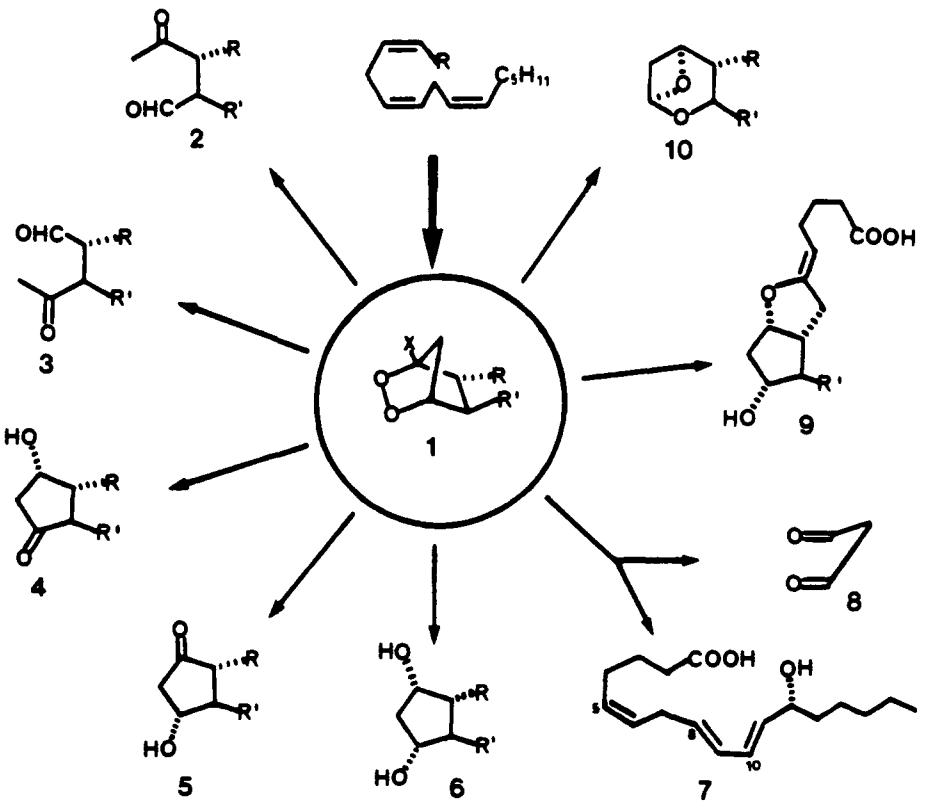
Contribution from the Department of Chemistry, Case Western Reserve University, Cleveland, Ohio 44106. *Received December 5, 1983*

Abstract: The prostaglandin (PG) endoperoxide nucleus, 2,3-dioxabicyclo[2.2.1]heptane, is three orders of magnitude less stable in aqueous vs. cyclohexane solution. Water-induced fragmentation of 2,3-dioxabicyclo[2.2.1]heptane-*1*-d_1 exhibits a deuterium kinetic isotope effect $k_H/k_D = 1.5$. Although rate-determining cleavage of a C–D bond occurs, deuterium is not lost to the protic solvent. Rather, the bridgehead deuterium migrates intramolecularly to the incipient methyl group of the levulinaldehyde product. Dimethyl sulfoxide (Me_2SO) also induces rapid decomposition of the PG endoperoxide nucleus at 37 °C. However, a different mechanism is suggested by $k_H/k_D = 3.3$ for the fragmentation reaction induced by Me_2SO. The PG endoperoxide PGH_2 [9α,11α-epidioxy-15(*S*)-hydroxy-5(*Z*),13(*E*)-prostadienoic acid] rearranges spontaneously at 37 °C in Me_2SO or aqueous solution to form levulinaldehyde derivatives, levuglandin (LG) E_2 [8(*R*)-acetyl-9(*R*)-formyl-12(*S*)-hydroxy-5(*Z*),10(*E*)-heptadecadienoic acid] and LGD_2 [9(*R*)-acetyl-8(*R*)-formyl-12(*S*)-hydroxy-5(*Z*),10(*E*)-heptadecadienoic acid]. The Me_2SO-induced fragmentation affords levuglandins in 70 to 80% yield according to ^{1}H NMR analysis of the decomposition product mixture while the yield of levuglandins in aqueous solution is about 22% at pH 7.9.

Prostaglandin (PG) endoperoxides, e.g., PGH_2 (**1a**), are pivotal intermediates in the biosynthesis of a vast array of natural products (Scheme I).[2] Most of the primary derivatives, **2a–10a**, exhibit potent physiological activities.[3] With the exception of **6a**, they

0002-7863/84/1506-6049$01.50/0

Scheme I



a: R = $CH_2CH{=}^{c}CH(CH_2)_3COOH$, R' = $CH{=}^{t}CHCH(OH)C_5H_{11}$, X = H b: R = R' = X = H

c: R = $CH_2CH{=}^{c}CH(CH_2)_3COOH$, R' = $CH{=}^{t}CHCH(OOH)C_5H_{11}$, X = H d: R = R' = H, X = D

are generated by rearrangements of the unusually reactive bicyclic peroxide nucleus **1b** of **1a**. Detailed study of these rearrangements is complicated, inter alia, by the availability of only minute quantities of **1a**, by the possibility of competing solvent-induced and enzymic processes, as well as by facile reactions of the primary derivatives. Thus, prostacyclin (**9a**) and thromboxane A_2 (**10a**) are rapidly hydrated ($t_{1/2}$ = 2 min and 30 s, respectively) under the conditions of their biosynthesis. Prostaglandin E_2 (**5a**), a β-hydroxy ketone, and the vinylogous β-hydroxy carbonyl compounds **2a–4a** readily undergo dehydration. Additional complication is caused by the possible existence of 15-hydroperoxy analogues of **2a–10a** which are produced directly from PGG_2 (**1c**), the 15-hydroperoxy precursor of PGH_2 (**1a**).[4]

One of the remarkable properties of PGH_2 is its extraordinary instability in the aqueous environment of its biosynthesis ($t_{1/2}$ = 5 min at 37 °C).[5] Furthermore, solvent-induced rearrangement of PGH_2 in aqueous solution at pH 8 is generally considered to afford 66% PGE_2 (**5a**) and 28% PGD_2 (**4a**), while 2% $PGF_{2\alpha}$ (**6a**) is produced by reductive cleavage of the peroxide bond.[5] However, our studies of a readily available[6] model, the PG endoperoxide nucleus **1b**, revealed an alternative rearrangement pathway.[7] Thus, **1b** gives 28% 3-hydroxycyclopentanone (**5b**) and 72% levulinaldehyde (**2b**) in D_2O at 40 °C.[7a] While the **1b** → **5b** disproportionation parallels the generation of PGD_2 (**4a**) and PGE_2 (**5a**) from PGH_2 (**1a**), the fragmentation of **1b** to give levulinaldehyde (**2b**) suggested a previously undetected rearrangement of **1a** to give levulinaldehyde derivatives **2a** and **3a**.

Table I. Solvent Effects for Decomposition of Endoperoxides **1b** and **11**

reaction solvent	dielectric constant[a]	relative rates	
		1b at 73 °C	**11** 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.

We now report a quantitative evaluation of a remarkable solvent effect on the stability of the PG endoperoxide nucleus **1b**. A thorough understanding of the mechanisms of solvent-induced rearrangement of **1b** is gleaned from kinetic and product studies with the monodeuterated peroxide **1d**. These model studies with

(1) Prostaglandin Endoperoxides. 14. For previous publications in this series see: (a) Zagorski, M. G.; Salomon, R. G. *J. Am. Chem. Soc.* **1983**, *105*, 1750. (b) **1982**, *104*, 3498 and references cited therein.

(2) For recent work see: van Dorp, D. A. In "Chemistry, Biochemistry and Pharmacological Activity of Prostanoids"; Roberts, S. M., Scheinmann, F., Eds.; Pergamon: New York, 1979; pp 233–242 and references cited therein.

(3) For reviews see: (a) Nicolaou, K. C.; Gasic, G. P.; Barnette, W. E. *Angew. Chem., Int. Ed. Engl.* **1978**, *17*, 293–378. (b) Samuelsson, B.; Goldyne, M.; Granstrom, E.; Hamberg, M.; Hammarstrom, S.; Malmsten, C. *Annu. Rev. Biochem.* **1978**, *47*, 997–1029.

(4) Raz, A.; Schwartzman, M.; Kenig-Wakshal, R. *Eur. J. Biochem.* **1976**, *70*, 89.

(5) (a) Nugteren, D. H.; Hazelhof, E. *Biochim. Biophys. Acta* **1973**, *326*, 448–461. (b) Nugteren, D. H.; Christ-Hazelhof, E. *Adv. Prostaglandin Thromboxane Res.* **1980**, *6*, 129–13. (c) Raz, A.; Kenig-Wakshal, R.; Schwartzman, M. *Biochim. Biophys. Acta* **1977**, *488*, 322–9.

(6) (a) Coughlin, D. J.; Salomon, R. G. *J. Am. Chem. Soc.* **1977**, *99*, 655–7. (b) Coughlin, D. J.; Brown, R. S.; Salomon, R. G. *Ibid.* **1979**, *101*, 1533–9. (c) Salomon, R. G.; Salomon, M. F. *Ibid.* **1977**, *99*, 3501–3. (d) Porter, N. A.; Gilmore, D. W. *Ibid.* **1977**, *99*, 3503–5. (e) Adam, W.; Eggelte, H. J. *J. Org. Chem.* **1977**, *42*, 3987–8. (f) Wilson, R. M.; Geiser, F. *J. Am. Chem. Soc.* **1978**, *100*, 2225–6.

(7) (a) Salomon, R. G.; Salomon, M. F.; Coughlin, D. J. *J. Am. Chem. Soc.* **1978**, *100*, 660–2. (b) Salomon, R. G.; Coughlin, D. J. *Ibid.* **1979**, *101*, 2761–3.

Table II. Temperature Dependence for Rate of Decomposition of **1b** in D_2O

T, °C	$10^3(1/T)$, K^{-1}	ln k	10^4k, s^{-1}
73.0	2.89	−3.05[a]	476[a]
48.4	3.11	−5.76[a]	31.5[a]
45.8	3.14	−6.07	23.2
37.0	3.22	−7.19	7.56
30.1	3.30	−8.13	2.95
21.0	3.40	−9.30	0.91

[a] Values extrapolated from the relationship in $k = -12338/T + 32.61$, see Figure 1.

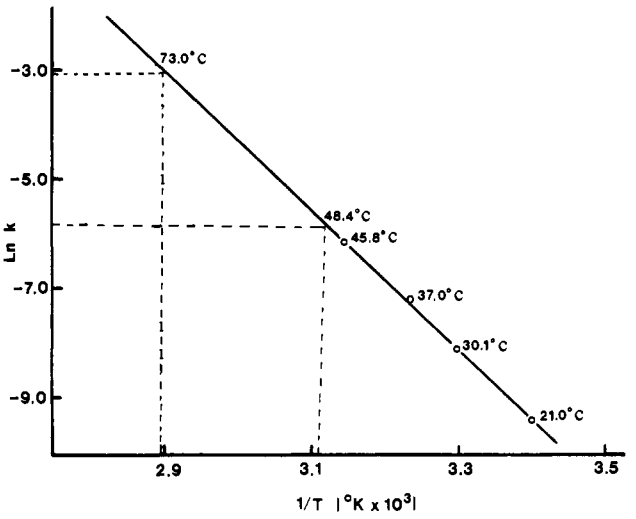


Figure 1. Temperature dependence for rate of decomposition of **1b** in D_2O.

simple bicyclic peroxides inspired and guided parallel studies on PGH_2. Thus, presumptive evidence is presented now for the formation of levulinaldehyde derivatives **2a** and **3a** from the prostaglandin endoperoxide PGH_2 (**1a**) by solvent-induced fragmentation. These new aldehyde products from PGH_2 were named[1] levuglandin (LG) E_2 (**2a**) and LGD_2 (**3a**) since these derivatives of levulinaldehyde are related to PGE_2 (**5a**) and PGD_2 (**4a**) respectively by aldol condensation.

Results and Discussion

Solvent Effects on Endoperoxide Stability. Thermal decomposition of the prostaglandin endoperoxide nucleus **1b** and the less strained homologue, 2,3-dioxabicyclo[2.2.2]octane (**11**), were

11

conveniently monitored by ^1H NMR in a variety of solvents at 73 and 130 °C, respectively (Table I).[7b] However, in aqueous solution the decomposition of **1b** is too rapid to measure by this method. Therefore the rate of spontaneous decomposition of **1b** in aqueous solution was determined at various temperatures over the range of 21 to 46 °C (Table II). These data show an excellent linear correlation ($r = 0.999$) expressed by the relationship $\ln k = -12338/T + 32.61$, as shown in Figure 1. Extrapolation to 73 °C gives $k = 4.76 \times 10^{-2}$ s^{-1}.

The data presented in Table I reveal two important facts. Protic solvents exert a profound destabilizing influence on the prostaglandin endoperoxide nucleus **1b** compared with the aprotic solvents of similar dielectric constant, and a similar effect is *not* observed for the closely homologous peroxide **11**. Thus, the rate of decomposition of **1b** 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 two orders of magnitude greater than expected for solvents of similar dielectric constant. This contrasts with the decomposition of **11** for which the rate in acetic acid or aqueous solution is not substantially

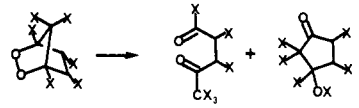
Table III. Solvent-Induced Rearrangement of **1d**[a]

solvent	product yields,[b] %				product ratios	
	2d	**2h**	**5d**	**5h**	**2d/2h**	**5d/5h**
H_2O	41	26	26	7.3	1.5	3.5
	43	26	24	7.6	1.6	3.2
CH_3COOH[c]					1.9	
					1.8	
Me_2SO	60	19	17	4	3.3	4.6

[a] $T = 37$ °C, [**1d**] ≃ 0.4 M. [b] Yields reported for **5d** and **5h** are the sums of the final yields of these products and their corresponding dehydration products (see ref 1) determined from ratios since overall yields are quantitative. [c] Since solvent peak overlaps with determination of **5d** and **5h**, only relative yields of **2d** and **2h** were determined in this solvent.

greater than expected for solvents of similar dielectric constant. To provide a mechanistic basis for understanding this unique behavior of the prostaglandin endoperoxide nucleus **1b**, kinetic and product studies were performed with 2,3-dioxabicyclo-[2.2.1]heptane-*1*-d_1 (**1d**) and 2,3-dioxabicyclo[2.2.1]heptane-*1,4,5,6,7,7*-d_6 (**1d$_6$**).

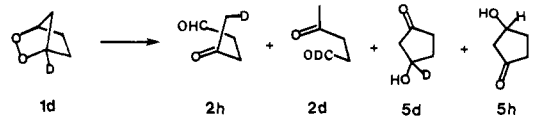
Kinetic Isotope Effects in Solvent-Induced Rearrangements of 2,3-Dioxabicyclo[2.2.1]heptane-d_6 (1d$_6$). Data for determination of deuterium kinetic isotope effects was obtained by measuring the rates of decomposition of the perprotio endoperoxide **1b** and the hexadeuterio endoperoxide **1d$_6$** in Me_2SO and D_2O solutions.



1b, X = H **2b**, X = H **5b**, X = H
1d$_6$, X = D **2d$_6$**, X = D **5d$_6$**, X = D

For decompositions in Me_2SO the rate constants for solvent-induced disappearance of **1** were measured at 37.0 °C by monitoring the appearance of **2**.[8] In Me_2SO the rate constant for disappearance of **1b** is $k_{-1} = 4.55 \times 10^{-4}$ s^{-1} and the product yields are **2b** (21%) and **5b** (79%) while the rate constant for disappearance of **1d$_6$** is $k_{-1} = 6.78 \times 10^{-5}$ s^{-1} and the product yields are **2d$_6$** (20%) and **5d$_6$** (80%). The rate constants for solvent-induced decomposition of **1** in D_2O solution were determined at 48.4 °C by monitoring the disappearance of **1**. In D_2O, the rate constant for disappearance of **1b** is $k_{-1} = 3.15 \times 10^{-3}$ s^{-1} while the rate constant for disappearance of **1d$_6$** is $k_{-1} = 7.83 \times 10^{-4}$ s^{-1}. Clearly the solvent-induced decomposition of **1b** is much faster than decomposition of **1d$_6$** in both Me_2SO and D_2O solutions. The data may be expressed as deuterium kinetic isotope effects $(k_H/k_D)_{-1} = 6.7$ and 4.0 in Me_2SO and D_2O, respectively, for disappearance of **1b** vs. **1d$_6$**.

Isotope Effects on Product Ratios from the Solvent-Induced Rearrangements of 2,3-Dioxabicyclo[2.2.1]heptane-d_1 (1d). The monodeuterated peroxide **1d** was dissolved in water, acetic acid, or dimethyl sulfoxide (Me_2SO) and kept at 37 °C. Although decomposition of undeuterated peroxide **1b** in water or Me_2SO is complete within an hour at 37 °C, the solutions of monodeuterated peroxide **1d** were heated at 37 °C for 24 h to ensure complete decomposition. For the rearrangement in acetic acid, ^1H NMR analysis of the solution after 2 days at 37 °C revealed incomplete decomposition. After 5 days at 37 °C no trace of **1d** could be detected. Yields of the four primary products **2d**, **2h**, **5d**, and **5h** presented in Table III were determined as described previously.[1a]



1d **2h** **2d** **5d** **5h**

(8) Since **1** decomposes by concurrent first-order pathways to **2** and **5**, the rate constant obtained by monitoring appearance of **2** or **5** is equal to the rate constant k_{-1} for disappearance of **1** and $k_{-1} = k_2 + k_5$ (see: Jencks, W. P. "Catalysis in Chemistry and Enzymology"; McGraw-Hill: New York, 1959; p 571).

Table IV. Distribution of Deuterium in Levulinaldehyde Products[a]

	product yields,[b] %					
solvent	CDH_2	CDH	CDH_2 + CDH	CHO	CDO	total D
H_2O	36	<1	36	39	61	97
	40	0	40	38	62	102
CH_3COOH	36	<1	36	34	66	102
	25	2	27	36	64	91
Me_2SO	12	11	23	23	77	100

[a] T = 37 °C, [**1d**] ≃ 0.4 M. [b] Yields reported for each type of deuterated group are percent of one atom of deuterium per molecule of levulinaldehyde product; e.g., $\%CDH_2 = (100)[CDH_2]/([CHO] + [CDO])$.

Control experiments showed that yields of levulinaldehyde (**2h**) and 3-hydroxycyclopentanone (**5h**) from solvent-induced decomposition of **1b** in Me_2SO or H_2O at 37 °C were essentially quantitative. Furthermore, no decomposition or interconversions of **2h** and **5h** were evident after prolonged heating for several days at 37 °C. Similarly, **2h** does not cyclize to **5h** in acetic acid under conditions resembling those for rearrangement of **1d**. Thus, **2h** was heated in acetic acid for 5 days at 45 °C and no change in the concentration of **2h** occurred and no **5h** or cyclopent-2-en-1-one was detectable by ^1H NMR.

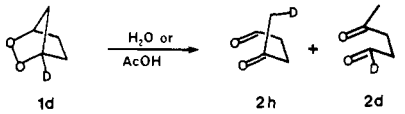
Deuterium Distribution in Levulinaldehyde Product. The distribution of deuterium in the levulinaldehyde products from **1d** presented in Table IV was determined as described previously.[1a] Studies of deuterium incorporation into levulinaldehyde (**2b**) by exchange or during rearrangement of **1b** which are presented in Table V were also conducted as described previously.[1a] In both Tables IV and V, deuterium at C-5, the methyl group of levulinaldehyde, is reported as CDH_2 while deuterium at C-2 or C-3, the methylene positions, is reported as CDH.

Since extensive deuterium incorporation into levulinaldehyde (**2b**) from CH_3COOD was observed, deuterium loss from hexadeuteriolevulinaldehyde ($\mathbf{2d_6}$) vide supra in CH_3COOH was examined under the same conditions. Thus, a solution of $\mathbf{2d_6}$ (0.31 M) in acetic acid was heated at 37 °C for 5 days, and then the amount of deuterium in the aldehydic, methyl, and methylene positions of $\mathbf{2d_6}$ was determined by ^2H NMR spectroscopy relative to 4-deuterio-1-methoxybenzene (0.20 M) as internal standard. Under these conditions, no deuterium loss from (protium incorporation into) $\mathbf{2d_6}$ was detected. Clearly hydrogen–deuterium exchange into levulinaldehyde is subject to a large deuterium kinetic isotope effect since loss of deuterium from $\mathbf{2d_6}$ is much slower than loss of protium from **2b**.

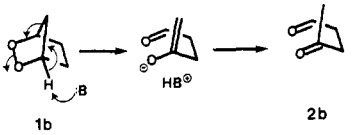
Effect of pH on the Rate of Decomposition of 2,3-Dioxabicyclo[2.2.1]heptane (1b). The decomposition of peroxide **1b** at 30.0 °C in pH buffered solution was monitored by measuring the decrease in the integral area of the peroxide methylene (C-5 and -6) proton absorption at δ 1.83–1.3 relative to the absorption of *tert*-butyl alcohol (δ 1.3–0.90) as internal standard. The δ values were assigned in reference to the sodium salt of 2,2-dimethyl-2-silapentane-5-sulfonate, a water-soluble silane reference. The rate constants found for the decomposition of endoperoxide **1b** are shown in Table VI.

Since the decomposition of 2,3-dioxabicyclo[2.2.1]heptane (**1b**) is especially rapid in protic solvents (Table I), the effect of pH on the aqueous decomposition of this endoperoxide is of interest. The results presented in Table VI show that, within experimental error, the pH of the aqueous medium had no effect on the rate of decomposition in aqueous solution. The insensitivity of the decomposition rate to differing pH conditions suggests that *protonation* of the peroxide oxygens is not involved in inducing the rearrangement of peroxide **1b** in aqueous solution.

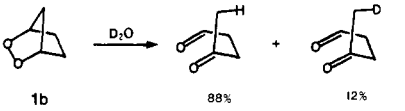
Mechanism of Solvent-Induced Fragmentation of 2,3-Dioxabicyclo[2.2.1]heptane in Water and Acetic Acid. Two features are noteworthy about the levulinaldehyde product from fragmentation of **1d** (see Table IV). One is the complete retention of deuterium. The second is the exclusive location of deuterium in the methyl group as in **2h** or aldehydic position as in **2d** but not the methylene positions. Thus, the total deuterium content of the levulinaldehyde



product from rearrangement of monodeuterated peroxide **1d** is, within the precision of the analytical method, one atom of deuterium per molecule. An intramolecular deuterium transfer seems to be occurring. Recently we presented evidence for a novel mechanism involving concerted cleavage of three bonds in order to account for large primary and secondary deuterium isotope effects observed for base-catalyzed fragmentation of **1b**.[1a]



However, a similar intermolecular mechanism involving transfer of a bridgehead deuteron to a molecule of protic solvent acting as base cannot be involved. Such an event would surely result in net loss of deuterium leading to deuterium free levulinaldehyde product owing to incorporation of protium from the conjugate acid of the solvent which necessarily contains both protium and deuterium. A minor contribution from an alternative mechanism involving intermolecular proton transfer is revealed by slight (12%) deuterium incorporation found (see Table V) in the methyl group of the levulinaldehyde product from rearrangement of **1b** in D_2O.



Under similar conditions, hydrogen–deuterium exchange of levulinaldehyde (**2b**) is only slight and occurs with a 4:1 preference in the methylene vs. the methyl groups. Since hydrogen–deuterium exchange of **2b** is extensive in acetic acid under similar conditions (see Table V), the observed distribution of deuterium in the methylene and methyl groups of the levulinaldehyde produced from **1b** in CH_3COOD (Table V) or from **1d** in CH_3COOH (Table IV) does not necessarily correspond with the distribution actually produced during the **1** → **2** rearrangement. However, a large kinetic deuterium isotope effect and a statistical factor of 3 decrease the rate of deuterium loss from **2h** compared to the rate of deuterium incorporation into **2b**, and little if any deuterium

Table V. Deuterium-Incorporation Studies[a]

			product yields,[b] %		product ratio
substrate and concn, M	solvent	time, days	CDH_2	CDH	CDH_2/CDH
levulinaldehyde (**2b**)					
0.74	D_2O	2	1	4	0.25
1.3	CH_3COOD	2	70	178	0.39
0.6[c]	Me_2SO	3	1	5	0.20
2,3-dioxabicyclo[2.2.1]heptane (**1b**)					
1.1	D_2O	1	12	2	6
1.0	CH_3COOD	3	44	98	0.45

[a] T = 37 °C. [b] Yields reported for each type of deuterated group are percent of one atom of deuterium per molecule of levulinaldehyde; e.g., $\%CDH_2 = (100)[CDH_2]/([CDO] + [CHO])$. [c] In the presence of 0.5 M Me_2CHOD.

Table VI. pH Dependence of the Decomposition of **1b** in Aqueous Solution at 30.0 °C

pH	10^4(rate constant), s^{-1}	pH	10^4(rate constant), s^{-1}
4.98	2.76 ± 0.16	8.13	2.93 ± 0.09
5.95	2.62 ± 0.06	9.00	2.83 ± 0.17
6.10	2.57 ± 0.11	9.90	2.70 ± 0.17
7.00	2.71 ± 0.26		

loss from **2h** was detected in CH_3COOH solution (see Table IV).

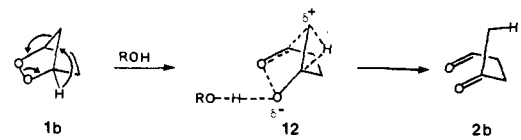
The solvent induced decomposition of $\mathbf{1d}_6$ is 4 times slower than decomposition of **1b** in D_2O. This large net deuterium kinetic isotope effect, $k(\mathbf{1b})/k(\mathbf{1d}_6) = KIE_6 = 4.0$, is probably the cumulative effect of a large primary KIE_α owing to deuterium substitution at C_α and several smaller secondary effects, $KIE_{\beta x}$, $KIE_{\beta n}$, $KIE_{\beta'}$, KIE_γ, and $KIE_{\gamma'}$, owing to the exo and endo deuterons at C_β and deuterium at $C_{\beta'}$, C_γ, and $C_{\gamma'}$.[1a] Thus cleavage

1

$$KIE_6 = KIE_\alpha \times KIE_{\beta x} \times KIE_{\beta n} \times KIE_{\beta'} \times KIE_\gamma \times KIE_{\gamma'}$$

of the C_α–H bond occurs during the rate-determining step for rearrangement of **1b** to **2b**, or **5b**, or both products of solvent-induced decomposition. Partial hydration of **2b**, which occurs in aqueous solution, complicates direct measurement of the yield of **2b** by NMR during decomposition of **1b**. The volatility of **2b** complicates accurate determination of the yield of **2b** by isolation from the aqueous reaction-product mixture. That solvent-induced rearrangements of **1b** to **2b** and **5b** are both susceptible to kinetic isotope effects was therefore demonstrated by examining isotope discrimination in the products isolated after completion of the decomposition of monodeuterated endoperoxide **1d**. In this case the isotope effects observed KIE = KIE_α/KIE_γ since molecules undergoing C_α–D cleavage have protium at C_γ whereas molecules undergoing C_α–H cleavage have deuterium at C_γ.[1a] It should be noted here that isotope discrimination in the formation of products does not necessarily give information on rate-determining steps. Thus, a common intermediate might be formed in a rate-determining step not involving C–D bond cleavage, and product isotope effects could arise during non-rate-determining partitioning of the intermediate to products. However, evidence was presented above supporting a rate-determining step for rearrangement of **1b** ($\mathbf{1d}_6$) which does involve C_α–H(D) bond cleavage. If a common intermediate is involved in the formation of the products **2b** and **5b**, then generation of the intermediate must involve rate-determining C_α–H(D) bond cleavage and both product isotope effects would be identical, i.e., **2d/2h = 5d/5h**.

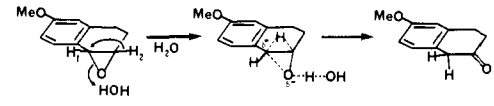
In aqueous solution the KIE revealed by the ratio of levulinaldehyde products formed by bridgehead C–H vs. C–D bond cleavage is given by the product ratio **2d/2h** = 1.5. This is much less than the KIE (3.3 to 3.8) observed[1] for the corresponding reactions catalyzed by tertiary amine[9] or carboxylate.[1b] A similar KIE = 1.8 is observed with **1d** in acetic acid solution. A novel mechanism involving 1,2-hydride shift accompanying concerted cleavage of three bonds can accommodate these observations. The transition state in such a mechanism could be stabilized by a hydrogen-bonding interaction with the protic solvent as depicted in **12**. This mechanistic hypothesis provides a rationale for the



1b 12 2b

R = Ac, H

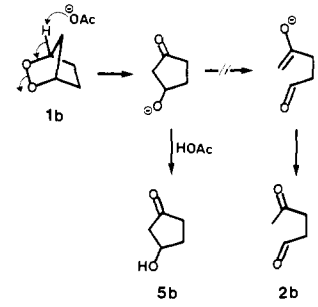
extraordinary instability of **1b** in protic solvents compared with aprotic solvents of similar dielectric constant (see Table I). It also explains why a comparable effect is *not* observed for the homologous peroxide **11** for which such a mechanism is not possible. A primary deuterium KIE of similar magnitude, $k_{H_2}/k_{D_2} = 1.59 \pm 0.03$, as well as an α secondary KIE, $k_{H_1}/k_{D_1} = 1.15 \pm 0.02$, is observed in the water-induced rearrangement of epoxide **13** to ketone **15**. A mechanism involving 1,2-hydride shift was postulated.[10] We suggest that the transition state in this mechanism could also be stabilized by a hydrogen-bonding interaction with the protic solvent as depicted in **14**.



13 14 15

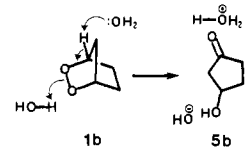
A much larger deuterium KIE (**5d/5h** = 3.3 ± 0.2) is observed for disproportionation of the prostaglandin endoperoxide nucleus **1d** to give 3-hydroxycyclopentanone (**5**) in aqueous solution (see Table III). Thus, the rate-determining steps of the solvent-induced fragmentation and disproportionation reaction of endoperoxide **1b** 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 **2b** and disproportionation product **5b**.

Mechanism of Solvent-Induced Disproportionation of 2,3-Dioxabicyclo[2.2.1]heptane (1b) in Water. Acetate-catalyzed rearrangement of **1b** is channeled by the presence of added acetic acid to favor disproportionation to 3-hydroxycyclopentanone (**5b**) over fragmentation to levulinaldehyde (**2b**).[1b] Large secondary



1b 5b 2b

deuterium isotope effects observed in the **1b** → **2b** rearrangement are not consistent with a mechanism in which the added acetic acid favors **5b** by intercepting a common β-ketoalkoxide intermediate.[1a] Rather the added acid probably provides an additional route to **5b** involving simultaneous bridgehead proton abstraction by acetate and protonation of the remote peroxide oxygen by acetic acid. A similar push–pull mechanism may be involved in the water-induced disproportionation of **1b** to give **5b** with water acting simultaneously as proton acceptor and proton donor.



1b 5b

Mechanism of Solvent-Induced Fragmentation of 2,3-Dioxabicyclo[2.2.1]heptane (1b) in Me_2SO. Listed in Table VII are the first-order rate constants, k_{obsd}, obtained by monitoring the appearance of levulinaldehyde products **2** from decomposition of the perprotio endoperoxide **1b** and the hexadeuterio endoperoxide $\mathbf{1d}_6$ in Me_2SO solution. The corresponding pseudo-first-order rate constants for decompositions of **1b** and $\mathbf{1d}_6$ in the presence of 0.015 M 1,4-diazabicyclo[2.2.2]octane (Dabco)[9] and 0.020 M Me_4NOAc[1b] as catalysts are also listed for comparison. The ratios of these k_{obsd}, which are rate constants for disappearance of **1**,[8]

(9) Zagorski, M. G.; Salomon, R. G. *J. Am. Chem. Soc.* **1980**, *102*, 2501–3.

(10) Gillilan, R. E.; Pohl, T. M.; Whalen, D. L. *J. Am. Chem. Soc.* **1982**, *104*, 4482.

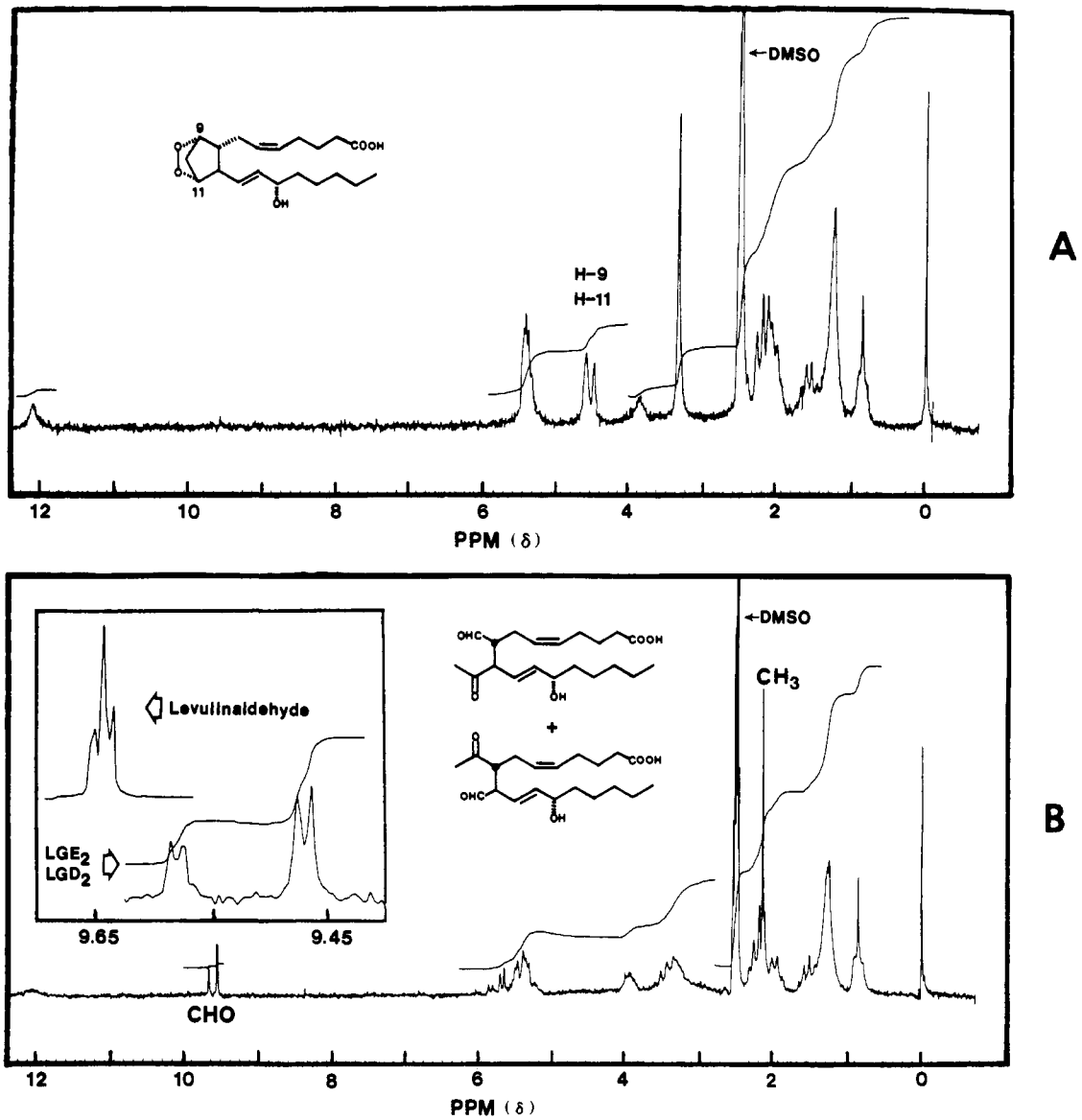
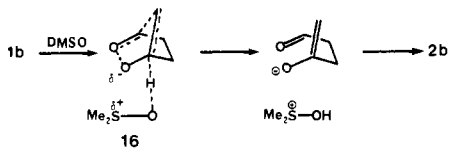


Figure 2. ^1H NMR spectra (100 MHz) in Me_2SO-d_6: A, PGH_2; B, products from decomposition of PGH_2 at 37 °C; inset in B, CH=O resonances of levuglandins (**2a** and **3a**) and levulinaldehyde (**2b**).

provide isotope effects $(k_H/k_D)_{-1}$ for disappearance of the endoperoxide. Isotope effects $(k_H/k_D)_2$ for fragmentation to levulinaldehyde (**2**) and $(k_H/k_D)_5$ for disproportionation of **1b** vs. **1d**$_6$ to 3-hydroxycyclopentanone (**5**) were calculated by using the relationship $k_{-1} = k_2 + k_5$ between the rate constant k_{-1} for disappearance of the endoperoxide and the rate constants k_2 and k_5 for appearance of the products in parallel (pseudo) first-order reactions. Thus, $k_2 = k_{-1}([2]_\infty/([2]_\infty + [5]_\infty))$ and $k_5 = k_{-1}([5]_\infty/([2]_\infty + [5]_\infty))$. The deuterium kinetic isotope effect $(k_H/k_D)_{-1} = 6.7$ for Me_2SO-induced disappearance of the endoperoxide **1b** vs. **1d**$_6$ is much larger than the corresponding isotope effect $KIE_6 = 4.0$ noted above for decomposition in D_2O, but it is similar to the effects observed for decompositions catalyzed by Dabco or Me_4NOAc. These observations show that the mechanism of solvent-induced fragmentation of **1b** in Me_2SO is different than that in the protic solvents acetic acid or water. Rather, Me_2SO may serve as a proton acceptor which induces a concerted three-bond cleavage process, depicted in **16**, which is analogous to that involved in the base-catalyzed fragmentations.[1b]



16

The isotope effect $(k_H/k_D)_2 = 7.0$ for fragmentation of the endoperoxide to levulinaldehyde is much larger than the product isotope effect **2d**/**2h** = 3.3 noted above for decomposition of the monodeuterated endoperoxide **1d**. The discrepancy reveals a large cumulative secondary deuterium isotope effect which accompanies bridgehead C–H bond cleavage during Me_2SO-induced fragmentation of the endoperoxide to levulinaldehyde. Thus deuterium substitution on C4 and C7 evidently produces large secondary kinetic isotope effects owing to cleavage of the C4–C7 bond which accompanies rate-determining cleavage of the bridgehead C–H bond. Previously we noted similar differences between $(k_H/k_D)_2$ and **2d**/**2h** for Dabco- and Me_4NOAc-catalyzed fragmentation of **1** to **2**.[1a] However, because we erroneously[8] presumed that the rate constant obtained by monitoring appearance of **2** was k_2 rather than k_{-1}, the isotope effects $(k_H/k_D)_{-1}$ for base-catalyzed disappearance of the endoperoxide were presumed to be the isotope effects $(k_H/k_D)_2$ for Dabco[9]- or Me_4NOAc[1b]-catalyzed fragmentation of **1b** vs. **1d**$_6$. The data in Table VII show that this erroneous presumption led to a small underestimation of $(k_H/k_D)_2$, and our conclusion that base-catalyzed fragmentation of **1d**$_6$ reveals large cumulative secondary kinetic isotope effects[1a] is now strengthened.

Levuglandins from PGH_2. A ^1H NMR spectrum of pure PGH_2 (**1a**) in Me_2SO-d_6, recorded at 19 °C by the FT technique <5 min after dissolution, reveals little decomposition (Figure 2A).

Table VII. Product Yields, Rate Constants, and Kinetic Isotope Effects for Decomposition of **1b** and $\mathbf{1d_6}$

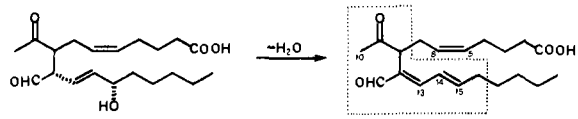
solvent (catalyst)	endoperoxide	product yields, %		$10^4 k_{obsd}$, s^{-1}	$(k_H/k_D)_{-1}$	$(k_H/k_D)_2$	$(k_H/k_D)_5$
		2b or $\mathbf{2d_6}$	**5b** or $\mathbf{5d_6}$				
C_6D_6 (Dabco)[a]	**1b**	80	20	7.37 ± 0.10	7.9	8.3	6.6
C_6H_6 (Dabco)[a]	$\mathbf{1d_6}$	76	24	0.935 ± 0.12			
$CDCl_3$ (Me_4NOAc)[b]	**1b**	51	49	6.06 ± 0.01	7.6	7.9	7.2
$CHCl_3$ (Me_4NOAc)[b]	$\mathbf{1d_6}$	49	51	0.802 ± 0.02			
Me_2SO[c]	**1b**	21	79	4.55 ± 0.04	6.7	7.0	6.6
Me_2SO[c]	$\mathbf{1d_6}$	20	80	0.678 ± 0.01			

[a] Dabco (0.015 M), reaction temperature 30.2 °C. [b] Me_4NOAc (0.020 M), reaction temperature 45.0 °C. [c] Reaction temperature 37.0 °C.

However, rapid decomposition ensues upon warming to 37 °C in the NMR probe affording a product mixture containing aldehyde resonances at δ 9.46 and 9.57, and no bridgehead H-9 and H-11 resonances of **1a** (Figure 2B). The only aldehyde derived from PGH_2 identified previously is malonaldehyde (**8**). The ^1H NMR spectrum of **8** in Me_2SO-d_6 shows aldehyde hydrogen resonances as a doublet at δ 8.46 (1 H, J = 10.5 Hz).[11] Both the high-field position and large hyperfine coupling contrast with the CHO resonances observed in Figure 2B. Thus, two new aldehyde-containing products are formed from PGH_2 upon decomposition in Me_2SO. Compelling evidence that these new products are levuglandins is provided by a comparison with a ^1H NMR spectrum of levulinaldehyde (**2b**) in Me_2SO-d_6. The CHO resonance for levulinaldehyde is a triplet owing to coupling with a neighboring CH_2 group. The corresponding resonances for the products from PGH_2 appear as doublets owing to coupling with neighboring CH groups as expected for LGE_2 (**2a**) and LGD_2 (**3a**) (see insert in Figure 2B). The aldehyde corresponding to thromboxane A_2 (**10a**), an acetal, also has a neighboring CH_2 group and would appear as a triplet. Furthermore, the characteristic upfield singlet at δ 2.12 owing to a methyl ketone group is clearly evident in Figure 2B but not Figure 2A. The Me_2SO-induced decomposition of PGH_2 produces levuglandins in about 70–80% yield.

Decomposition of pure PGH_2 was also examined directly by ^1H NMR in pH 7.9 buffered D_2O. Sharp aldehyde resonances were *not* observed although a broad resonance was present in the CHO hydrogen region. Since levulinaldehyde derivatives might form hydrates in aqueous solution, the decomposition reaction mixture was acidified to pH 3 and extracted with diethyl ether. The solution was dried over sodium sulfate for 15 h at 20 °C. The ^1H NMR spectrum in $CDCl_3$ of the residue after removal of the ether solvent showed two sharp resonances at δ 9.74 and 9.49 which were assigned to levuglandins formed in 22% yield from decomposition of PGH_2 in aqueous solution. These same two sharp resonances are observed if the solvent is removed from the rearrangement of PGH_2 in Me_2SO and the residue is dissolved in $CDCl_3$.

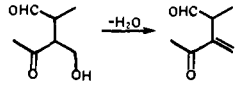
The new aldehyde products from PGH_2, presumed to be LGE_2 and LGD_2, readily decompose to afford, inter alia, a less polar aldehyde which exhibits intense ultraviolet absorption and ^1H NMR resonances at δ 9.61 (1 H), 6.0–6.2 (2 H), and 5.1–5.3 (2 H). This less-polar aldehyde was formed from a rearrangement product mixture from PGH_2 containing about 70% of LGE_2 and LGD_2 upon standing in $CDCl_3$ solution or upon removal of the Me_2SO solvent followed by attempted purification by HPLC on Partisil. In the latter experiment a polar fraction was obtained which closely resembled the original mixture of aldehydes (sharp ^1H NMR resonances at δ 9.74 and 9.49 in $CDCl_3$) while a less polar fraction was obtained which contained the new aldehyde (see Experimental Section). The low-field vinyl resonances, intense UV activity, and decreased polarity of this aldehyde all suggest a dehydration product such as anhydro-LGE_2 (**17**). Neither the



LGE_2 (2a) 17

decreased polarity nor the ^1H NMR resonance at δ 6.0–6.2 (2 H) is expected for Δ^{12}-13,14-dihydro-LGD_2 which might be formed by a prototropic shift analogous to a known isomerization of PGD_2 (**4a**).[12] Since PGH_2 is available only in minute quantities from bioconversion of arachidonic acid, thorough chemical characterization of these new aldehyde products is hampered not only by their instability but also by their limited availability from natural sources. We are examining total synthesis as a practical alternative source of pure levuglandins to facilitate structure proof and biological evaluation.

It seems highly likely that levuglandins have major significance for human health. These molecules contain multiple sites of electrophilic reactivity which allow covalent binding with biological nucleophiles. Levuglandins, especially LGD_2 (**3a**), have a striking structural and functional resemblance with botryodiplodin (**18**), a natural antibiotic and antitumor mold metabolite[13] which probably owes its biological activity to such covalent binding.[14] Furthermore, a potent electrophilic center can be activated by dehydration of the aldol **18** to produce the Michael acceptor **19**.

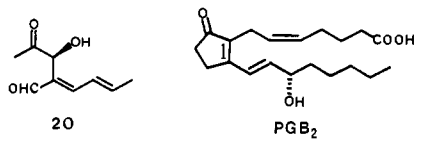


18 19

Even more remarkable is the structural analogy between anhydro-LGE_2 (**17**) and the antibiotic natural product (+)-(*R*)-avellaneol (**20**) which also exhibits intense UV activity with λ_{max}^{EtOH} 278 (ε 19 500).[15] It has not escaped our attention that this λ_{max} is very similar to that reported[16] for PGB_2, and it is conceivable that assays for prostaglandins based on conversion to, and UV detection of, PGB_2 would be confused by the presence of anhydro-LGE_2 (**17**). As for **18**, the biological activity of **20** probably results from alkylation of biological nucleophiles by this polyelectrophile. Michael addition of thiols to α,β-unsaturated carbonyl compounds is often associated with antitumor activity.[14] A variety of 1,4-dicarbonyl compounds including α-polygodial (**21**) and

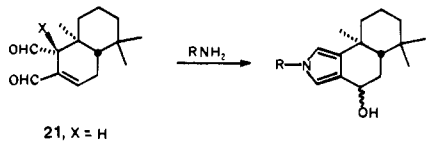
(11) Methylene hydrogen resonances appear as a triplet at δ 5.42 (2 H, J = 10.5 Hz) and the spectrum is very similar to that reported for **8** in acetone-d_6: Brown, R. S. *J. Am. Chem. Soc.* **1977**, *99*, 5497–9. The unusually high field position of the aldehyde resonance is a result of rapid equilibration with the enol tautomer.

(12) Stehle, R. G. *Methods Enzymol.* **1982**, *86*, 452.
(13) (a) Gupta, R. S.; Chandran, R. R.; Divekar, P. V. *Indian J. Exp. Biol.* **1966**, *4*, 152–3. (b) Arsenault, G. P.; Althaus, J. R.; Divekar, P. V. *J. Chem. Soc., Chem. Commun.* **1969**, 1414–5. (c) McCurry, P.M., Jr.; Abe, K. *Tetrahedron Lett.* **1973**, 4103–6. (d) Mukaiyama, T.; Wada, M.; Hanna, J. *Chem. Lett.* **1974**, 1181. (e) Nakagawa, F.; Kodama, K.; Furuya, K.; Naito, A. *Agric. Biol. Chem.* **1979**, *43*, 1597–8.
(14) (a) Kupchan, S. M. *Fed. Proc., Fed. Am. Soc. Exp. Biol.* **1974**, *33*, 2288–95. (b) Moore, H. W. *Science* **1977**, *197*, 527–32. (c) Brooks, B. R.; Klamerth, O. L. *Eur. J. Biochem.* **1968**, *5*, 178–182.
(15) Ananthasubramanian, L.; Carey, S. T.; Nair, M. S. R. *Tetrahedron Lett.* **1978**, 3527.
(16) Bergstrom, S.; Rhyge, R.; Samuelsson, B. *Proc. Natl. Acad. Sci, U.S.A.* **1974**, *71*, 3400.

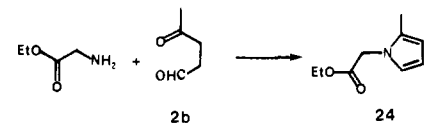


20 PGB_2

warburganal (**22**) are insect antifedents.[17] Formation of covalent adducts, e.g., **23**, with primary amines is associated with their biological activity.[18] In a model study we found that levulinaldehyde forms a covalent adduct, the pyrrole **24**, with glycine ethyl ester in aqueous solution at room temperature. Similar

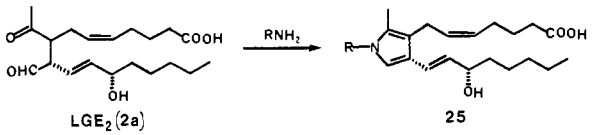


21, X = H
22, X = OH 23



2b 24

adducts, e.g., **25**, are one of the many possible modes of covalent binding of levuglandins with biological nucleophiles which could have important biological consequences.[19] Besides possible biological actions of levuglandins, their production could compete with and thus curtail the biosynthesis of prostaglandins (**4a–6a**), prostacyclin (**9a**), or thromboxane A_2 (**10a**). It is interesting in this regard that topical application of Me_2SO is widely used to reduce pain and inflammation,[20] phenomena often associated with prostaglandins.[21]



LGE_2 (2a) 25

The discovery that LGE_2 and LGD_2 are major products from solvent-induced decomposition of PGH_2 confirms the predictive power of model studies with **1b** which further suggest that levuglandins may also be produced in various catalyzed reactions of PGH_2 (**1a**).[1] Clearly, future studies on enzyme and other catalyzed transformations of PGH_2 must explore the possibility that the formation of levuglandins is involved. Numerous reports document the formation of unidentified derivatives from PGH_2 or warn that such products might not be chromatographically distinguished readily from prostaglandins.[22]

(17) (a) Kubo, I.; Lee, Y.-W.; Pettei, M. J.; Pilkiewicz, F.; Nakanishi, K. *J. Chem. Soc., Chem. Commun.* **1976**, 1013. (b) Barnes, C. S.; Loder, J. W. *Aust. J. Chem.* **1962**, *15*, 322. (c) Brooks, C. J.; Draffan, G. H. *Tetrahedron* **1969**, *25*, 2887. (d) Kubo, I.; Miura, I.; Pettei, M. J.; Lee, Y.-W.; Pilkiewicz, F.; Nakanishi, K. *Tetrahedron Lett.* **1977**, 4553.

(18) D'Ischia, M.; Prota, G.; Sodano, G. *Tetrahedron Lett.* **1982**, *23*, 3295.

(19) Covalent binding of unidentified arachidonic acid metabolites, apparently derived from prostaglandin endoperoxides, with tissue macromolecules has been detected during prostaglandin biosynthesis: (a) Eling, T. E.; Wilson, A. G. E.; Chaudhari, A.; Anderson, M. S. *Life Sci.* **1977**, *21*, 245–51. (b) Anderson, M. W.; Crutchley, D. J.; Chaudhari, A.; Wilson, A. G. E.; Eling, T. E. *Biochim. Biophys. Acta* **1979**, *573*, 40–50.

(20) (a) Jacob, S. W.; Rosenbaum, E. E.; Wood, D. C., Eds. "Dimethyl Sulfoxide: Volume 1. Basic Concepts of DMSO"; Marcel Dekker: New York, 1971. (b) Steinberg, A. *Ann. N.Y. Acad. Sci.* **1967**, *141*, 532–550. (c) Persky, L.; Stewart, B. H. *Ibid.* **1967**, *141*, 551–554. (d) Zuckner, J.; Uddin, J.; Gantner, G. E., Jr. *Ibid.* **1967**, *141*, 555–9. (e) Matsumoto, J. *Ibid.* **1967**, *141*, 560–8. (f) Hohn, H.; Laudahn, G. *Ibid.* **1967**, *141*, 506–16.

(21) (a) Kuehl, F. A., Jr.; Humes, J. L.; Egan, R. W.; Ham, E. A.; Beveridge, G. C.; Van Arman, C. G. *Nature (London)* **1977**, *265*, 170–3. (b) Vane, J. R. *Adv. Prostaglandin Thromboxane Res.* **1976**, *2*, 791–801. (c) Greaves, M. W. *Adv. Prostaglandin Res.* **1976**, *3*, 293–302. (d) Weissmann, G.; Smolen, J. E.; Korchak, H. *Adv. Prostaglandin Thromboxane Res.* **1980**, *8*, 1637–53.

(22) (a) Lands, W.; Lee, T.; Smith, W. *Ann. N.Y. Acad. Sci.* **1971**, *180*, 107–122. (b) Dunn, M. J.; Howe, D.; Harrison, M. *J. Clin. Invest.* **1976**, *58*, 862–870. (c) Wong, P. Y.-K.; Majeska, R. J.; Wuthier, R. E. *Prostaglandins* **1977**, *14*, 839–51.

Experimental Section

General. NMR and mass spectroscopy, chromatography, and collection of kinetic data were performed as described previously.[1a] Ram seminal vesicles were stored at −80 °C in a Revco ULT 1275 freezer and sonicated with a Brinkman PT 10/35 Polytron homogenizer. Ram seminal vesicle microsomes were collected with a Beckman L8-55 ultracentrifuge. Preparative high-pressure liquid chromatography was performed on a Waters Associates System consisting of a Waters M-6000A solvent delivery system, a Waters U6K injector, a Waters μ porasil column (10 μM particles, 3.9 mm (i.d.) × 30 cm), and a Waters R-401 differential refractometer.

Materials. Besides those materials described in the preceding paper[1a] malonaldehyde was prepared by the method of Huttel.[23] Water was purified by passage through a reverse osmosis membrane to remove all organic and particulate matter followed by a careful distillation under flowing N_2 with partial condensation. The water had a resistivity of 9 MΩ cm^{-1}, virtually free from contaminants. Dimethyl sulfoxide (Me_2SO) was purified by stirring over BaO for 1 day followed by distillation. Diethyl ether was dried by distillation from $LiAlH_4$. Ether used for extractions was distilled to remove nonvolatile impurities. Ethyl acetate and chloroform-d_1 were refluxed over P_2O_5 for 4 h followed by distillation. Hexane was stirred over concentrated H_2SO_4 for 1 day at room temperature and then refluxed over fresh H_2SO_4 for 1 day, washed with water, saturated $NaHCO_3$, water, dried over anhydrous $CaCl_2$, refluxed over potassium 1 day, and distilled. Benzene was shaken with cold concentrated H_2SO_4 followed by water, 1% NaOH, and water, dried over anhydrous $CaCl_2$, and distilled from potassium. All *cis*-5,8,11,14-eicosatetraenoic (arachidonic) acid was obtained from United States Biochemical Corp. The fatty acid was chromatographed on a column of activated silicic acid (Bio-Rad Corp. Bio-Sil A 100–200 mesh) with use of hexane/ether (95/5, v/v) as eluent with fractions taken under an argon blanket. Pure fatty acid was stored as a 20% solution in dry benzene containing 10 μM BHT under nitrogen at −20 °C. *p*-(Hydroxymercuri)benzoic acid sodium salt, phenol, and Me_2SO-d_6 (sealed ampules) were obtained from Aldrich and used without further purification.

Preparative Biosynthesis of PGH_2 (1a). Microsomes from sheep seminal vesicles were prepared as described previously.[24] Microsomes were resuspended in 10 mM potassium phosphate pH 7.4 buffer to a concentration of 2 g of vesicular tissue/mL. The suspended microsomes were immediately incubated with arachidonic acid by a modification of the method of Graff.[25] The suspended microsomes from 45 g of tissue were added to 128 mL of 0.1 M sodium phosphate pH 7.8 buffer containing 0.5 mM *p*-(hydroxymercuri)benzoate and 1.0 mM phenol. The mixture was equilibrated at 25 °C for 3 min. Arachidonic acid was dissolved in a small aliquot of ethanol and added to the incubation medium to initiate the reaction. The final concentration of the fatty acid was 0.53–0.55 μmol/mL. The reaction mixture was shaken vigorously by hand and oxygen gas (200 mL/min) was introduced via a gas-dispersion tube. After 2 min the reaction was terminated by the addition of enough 6 M HCl to the mixture to give pH 3. The reaction mixture was then rapidly extracted twice with equal volumes of cold (4 °C) diethyl ether. The combined organic extracts were dried over 20 g of anhydrous magnesium sulfate for 30 min at 4 °C. Following filtration the extract was cooled to −80 °C. The solvent volume was reduced to 50 mL with use of a high-vacuum rotary evaporator without heating of the rotary flask. To the concentrated extract was added dry hexane (20 mL) and anhydrous magnesium sulfate. The extract was filtered, using dry diethyl ether to rinse, into a silanized evaporation flask. The solvent volume was reduced to 5 mL, and the above drying procedure was repeated before final concentration of the extract. PGH_2 was purified by HPLC by using the procedure described previously[26] adapted to an analytical column. Once the crude extract has been thoroughly dried it can be kept at room temperature for several hours without noticeable decomposition. For this reason, HPLC purification was carried out at ambient temperature. The column was equilibrated with a 60/40 mixture by volume of dry ethyl acetate/hexane at a flow rate of 1 mL/min. Typically, 20 mg of the crude extract dissolved in the mobile phase at a concentration of 1 mg/5 μL was injected onto the column. PGH_2 was the last eluting substance, exiting the column 7.5 min after injection. The portion of the eluate-containing PGH_2 was collected in a silanized evaporation flask and concentrated by rotary evaporation at 20 mm without warming of the flask. Last traces of solvent were removed under high vacuum with the flask kept at 0 °C. PGH_2 was stored in dry $CDCl_3$

(23) Huttel, R. *Chem. Ber.* **1941**, *74*, 1825.

(24) Marnett, L. J.; Bienkowski, M. J.; Pagels, W. R. *J. Biol. Chem.* **1979**, *254*, 5077.

(25) Graff, G. *Methods Enzymol.* **1982**, *86*, 376.

(26) Porter, N. A.; Byers, J. D.; Holden, K. M.; Menzel, D. B. *J. Am. Chem. Soc.* **1979**, *101*, 4319.

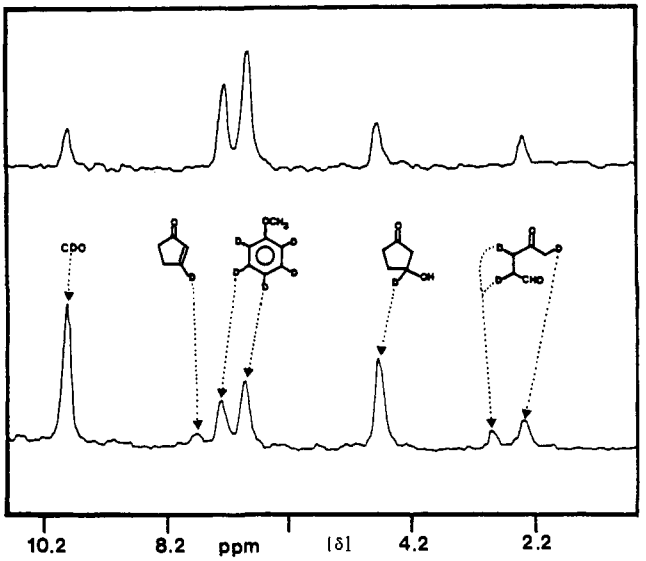


Figure 3. 15.36-MHz ^2H NMR spectra of reaction product mixture (top) from rearrangement of **1d** in aqueous solution and for comparison (bottom) from Me_4NOAc-catalyzed rearrangement of **1d** in CCl_4 both with **26** as internal standard.

in an NMR tube at −20 °C under which conditions it is stable for several months.

Rearrangements of 1-Deuterio-2,3-dioxabicyclo[2.2.1]heptane (1d). Method of Rearrangement. For all studies, the peroxide **1d** (12–14 mg, 0.12–0.14 mmol) stored in Freon 11 in a 5-mm NMR tube was concentrated to dryness under reduced pressure (110 mm, 5 °C, under a dry N_2 bleed) by placing the NMR tube containing the peroxide and Freon 11 inside a vacuum desiccator at 5 °C[27] and capping the tube with a serum cap equipped with a syringe needle piercing the cap. When only crystalline peroxide remained in the NMR tube, 0.3 mL of each solvent (vide infra) was added to the NMR tube. The solution was immediately mixed thoroughly by shaking and then heated in a thermostated oil bath at 37.0 °C. For the rearrangements in H_2O and Me_2SO the solutions were heated for 1 day, whereas for the rearrangement in acetic acid, the solution was heated for 5 days.[28]

Analysis of Products from Rearrangement in Aqueous Solution. A solution (10.0 mL) was prepared volumetrically containing 2,3,4,5,6-pentadeuterio-1-methoxybenzene (**26**)[1a] (11 μL, 11 mg, 0.099 mmol) in CCl_4. The contents of the NMR tube containing **1d** in highly purified H_2O after 1 day at 37 °C were poured into a separatory funnel and saturated with NaCl. The aqueous layer was extracted with ether (8 × 8 mL) and the combined ether extracts were dried over anhydrous magnesium sulfate. The ether was removed by rotary evaporation (20 mm, 20 °C), and the residue was dissolved in 0.3 mL of a 0.010 M **26** in CCl_4 solution and transferred to a 5-mm NMR tube. The NMR tube was fitted with a coaxial inner cell containing acetone-d_6 which was used as an internal ^2H lock on the Varian XL-200 NMR spectrometer.

The concentrations of the products, levulinaldehyde (**2h**) and 3-hydroxycyclopentanone (**5h**), from decomposition of **1d** were determined relative to the internal standard **26** by ^1H NMR (200 MHz) spectroscopy by using ratios of integrated areas of the peaks at δ 9.72 (s, 1 H), 4.52 (m, 1 H), and 3.74 (s, 3 H) for the two products (**2h** and **5h**) and **26** (0.010 M), respectively (Table III). Next, the acetone-d_6 was removed from the coaxial inner cell, and the concentration of the deuterated products **2d** and **5d** was determined relative to the internal standard **26** by ^2H NMR (15.36 MHz) spectroscopy (Figure 3). The ratios of the integrated areas of the ^1H-decoupled ^2H NMR signals at δ 9.82 (s, 1 ^2H), 4.59 (s, 1 ^2H), 2.72 (s, 1 ^2H), 2.18 (s, 1 ^2H), and 7.27 (s, 2 ^2H), 6.90 (s, 3 ^2H) which corresponded to the aldehydic absorption of **2d**, α to OH resonance of **5d**, methylene resonance of levulinaldehyde, methyl resonance of levulinaldehyde, and **26** were used to calculate the deuterium content and distribution in the levulinaldehyde product (Table IV) and the concentrations of **2d** and **5d** (Table III).

Analysis of Products from Rearrangement in Me_2SO Solution. A solution (1.0 mL) was prepared volumetrically containing 4-deuterio-1-methoxybenzene (**27**)[1b] (6.6 μL, 6.5 mg, 0.060 mmol) in dry Me_2SO. Approximately 2–4 drops of the above solution was added to the 5-mm NMR tube containing the products from decomposition of **1d** in Me_2SO. The NMR tube was fitted with a coaxial inner cell containing acetone-d_6 which provided an internal ^2H lock for use on the XL-200 NMR spectrometer. The concentrations of the two products, levulinaldehyde (**2h**) and 3-hydroxycyclopentanone (**5h**), from the decomposition of **1d** were determined relative to the internal standard **27** by ^1H NMR (200 MHz) spectroscopy by using ratios of integrated areas of the peaks at δ 9.62 (s, 1 H), 4.36 (m, 1 H), and 7.25 (br d, 2 H), and 6.89 (dd, 2 H) for the products (**2h** and **5h**) and **27**, respectively (Table III). The intense solvent (Me_2SO) peak at δ 2.55 (s, 6 H) was irradiated at a low power output of 20 W, thus enabling detection of the weaker signals of the products **2h**, **5h**, and internal standard **27**.[29]

Next, the acetone-d_6 was removed from the coaxial inner cell, and the concentrations of the deuterated products **2d** and **5d** were determined relative to internal standard **27** by ^2H NMR (15.36 MHz) spectroscopy. The ratios of the integrated areas of the ^1H-decoupled ^2H NMR signals at δ 8.64 (s, 1 ^2H), 3.38 (s, 1 ^2H), 1.58 (s, 1 ^2H), 1.14 (s, 1 ^2H), and 5.95 (s, 1 ^2H) which corresponded to signals for the aldehydic resonance of **2d**, α to OH resonance of **5d**, methylene resonance of levulinaldehyde, methyl resonance of levulinaldehyde, and **27** were used to calculate the deuterium content and distribution in the levulinaldehyde product (Table IV) and the concentrations of **2d** and **5d** (Table III).[30]

Analysis of Products from Rearrangement in Acetic Acid Solution. A solution (1.0 mL) was prepared volumetrically containing 4-deuterio-1-methoxybenzene (**27**) (6.6 μL, 6.5 mg, 0.060 mmol) in acetic acid. Approximately 2–4 drops of the above solution was added to the 5-mm NMR tube containing the products from decomposition of **1d** in acetic acid. The concentration of the product levulinaldehyde (**2h**) was determined by ^1H NMR (60 and 100 MHz) spectroscopy in the continuous wave mode by using ratios of integrated areas of the peaks at δ 9.62 (s, 1 H), 7.27 (br d, 2 H), and 6.90 (dd, 2 H) which corresponded to peaks for **2h** and internal standard **27**, respectively (Table III). Next, the concentration of **2d** relative to **27** was determined by ^2H NMR (15.36 MHz) spectroscopy. The ratios of the integrated areas of the ^1H-decoupled ^2H NMR signals at δ 9.44 (s, 1 ^2H), 2.45 (s, 1 ^2H), 1.89 (s, 1 ^2H), and 6.67 (s, 1 ^2H) which corresponded to signals for the aldehydic absorption of **2d**, methylene absorption of levulinaldehyde, methyl absorption of levulinaldehyde, and **27** were used to calculate the deuterium content and distribution in the product levulinaldehyde (Table IV) and the concentration of **2d** (Table III).[31]

Kinetics of Peroxide Decompositions. (a) 2,3-Dioxabicyclo[2.2.1]heptane (1b) and 2,3-Dioxabicyclo[2.2.1]heptane-*1,4,5,6,7,7*-d_6 ($1d_6$) in Me_2SO at 37.0 °C. A solution (5.0 mL) was prepared volumetrically containing *p*-dichlorobenzene (77 mg, 0.52 mmol, 0.11 M) and benzene-d_6 (31 μL, 30 mg, 0.35 mmol, 0.069 M) in dry Me_2SO. The appearance of **2h** from **1b** was monitored by ^1H NMR (60 MHz) at 37.0 °C by integration of the aldehydic proton of **2h** (δ 9.62 (s, 1 H)) and the inert internal standard *p*-dichlorobenzene (δ 7.48 (s, 4 H)). The appearance of $2d_6$ from $1d_6$ was monitored by ^2H NMR (15.36 MHz) at 37.0 °C by integration of the aldehydic deuteron resonance of $2d_6$ (δ 8.64 (s, 1 ^2H) and the inert internal standard benzene-d_6 (δ 7.27 (s, 6 ^2H)). From the ratio of the integral area of the aldehydic NMR signal to that for the internal standard, the concentration of **2b** or $2d_6$ was determined at each time interval. The concentration of **2b** or $2d_6$ at time = ∞, $[2b]_\infty$ or $[2d_6]_\infty$, was extrapolated from the graphical representations of [**2b**] or [$2d_6$] vs. time. Since the decomposition of **1b** or $1d_6$ is first order, the following relation for an exponential first-order decay[32]

$$([2]_\infty - [2]) = ([2]_\infty - [2]_0)e^{-tk_{obsd}}$$

$$\ln([2]_\infty - [2]) = \ln([2]_\infty - [2]_0) = -tk_{obsd}$$

$$\ln(([2]_\infty - [2])/([2]_\infty - [2]_0)) = tk_{obsd}$$

(27) The reaction was performed in a cold room kept at 5 °C.

(28) The rate of solvent-induced fragmentation of **1d** to **2d** at 37 °C in acetic acid ($t_{1/2} \sim 2$ days) is considerably slower than that in water ($t_{1/2} \sim 15$ min) and Me_2SO ($t_{1/2} \sim 25$ min).

(29) A control experiment on products from a decomposed, concentrated (1.0 M) sample of **1d** in Me_2SO showed that measurements of the concentration of the products by ^1H NMR (60 MHz) spectroscopy in the continuous wave mode and in the FT mode with irradiation of the Me_2SO peak on the XL-200 spectrometer agreed ±5%. Thus, irradiation of the Me_2SO signal at δ 2.55 presumably did not affect (possibly by saturation) measurements of the products or internal standard signals at δ 9.62, 4.36, 7.25, and 6.89.

(30) The solvent (Me_2SO) also gave a ^2H NMR absorption at δ 2.37 (s) which was shown to be from natural abundance deuterium (0.015%).

(31) The solvent (CH_3COOH) also gave a ^2H NMR absorption at δ 1.86 (s) which overlapped with the methyl ^2H NMR resonance of levulinaldehyde. Thus, we separately determined that neat acetic acid contained ca. 0.0096 M natural abundance deuterium in the methyl substituent and, accordingly, subtracted this from the ^2H NMR signal at δ 1.89–1.86.

(32) Gilliom, R. D. "Introduction to Physical Organic Chemistry"; Addison-Wesley: Reading, Mass., 1970; p 113.

(where $[2]_\infty$ is the final concentration for the product levulinaldehyde, [2] is the concentration of levulinaldehyde at time t, $[2]_0$ is the initial concentration of levulinaldehyde at $t = 0$) was used to evaluate the rate constant k_{obsd} which is the rate constant k_{-1} for disappearance of the endoperoxide **1**.[8] Alternatively, since it was often difficult to obtain an accurate value for $[2]_\infty$, k_{obsd} was evaluated with a computer program which changed $[2]_\infty$ in small increments until a value for k_{obsd} was reached giving a minimum in the root-mean-square deviation between observed and calculated values of [2].[1] The pseudo-first-order rate constants for Dabco- or Me_4NOAc-catalyzed decomposition of **1** were determined similarly and are reported in Table VII. Product yields, also reported in Table VII, were calculated from $[2]_\infty$ and $[5]_\infty$.

(b) 2,3-Dioxabicyclo[2.2.1]heptane (1b) in D_2O. These kinetics were performed by ^1H NMR (CW mode-60 MHz and FT mode-100 MHz) by monitoring the decrease of **1b** relative to the inert internal standard *tert*-butyl alcohol. A solution (5.0 mL) was prepared volumetrically from *tert*-butyl alcohol (50 μL, 39 mg, 0.53 mmol, 0.11 M) in D_2O. The probe of the A-60-A NMR spectrometer was preheated to 21.0, 30.1, or 37.0 °C by careful adjustment of the instrument's variable temperature controller and careful inspection of the probe's temperature with a thermometer (Brooklyn P-M, 6N328, 76 mm × 1 mm. ±0.2 °C) specially designed to fit inside the NMR probe. After careful tuning of the NMR spectrometer, 0.4 mL of the 0.11 M aqueous *tert*-butyl alcohol solution (vide infra) was added to a clean 5-mm NMR tube containing 40 mg (0.40 mmol) of **1b**. The aqueous endoperoxide solution was immediately mixed thoroughly by shaking the tube and placed into the NMR probe at the specific temperature. A timer was started, and data were acquired by recording the time, then immediately integrating the resonances for the endoperoxide (**1b**) methylene protons (C_5–C_6) at δ 1.83–1.30 (s, 2 H) (relative to sodium 2,2-dimethyl-2-silapentane-5-sulfonate) and the internal standard, *tert*-butyl alcohol, at δ 1.30–0.90 (s, 9 H). Integrals were taken as often as required which depended upon how fast decomposition of **1b** was occurring. For decomposition of **1b** at 45.8 °C, the XL-100 NMR spectrometer was used. From the ratio of the integrated areas of the peaks for **1b** and internal standard *tert*-butyl alcohol (0.11 M), the concentration of **1b** ([**1b**]) was calculated for each time. Graphs were constructed of the natural logarithm of [**1b**] vs. time, and the straight line obtained gave a slope equal to the rate constant (k) for disappearance of **1b** at each temperature (Table II). Using the Arrhenius equation[32]

$$k = Ae^{-E_a/RT}$$

or

$$\ln k = \ln A = E_a/RT$$

a plot of ln k vs. $1/T$ (K) was constructed to give a straight line (Figure 1).

(c) *exo,exo*-5,6-Diprotio-2,3-dioxabicyclo[2.2.1]heptane-*1,4,5,6,7,7*-d_6 ($1d_6$) in D_2O. These kinetics were performed by 200 MHz FT ^1H NMR monitoring of the decrease of $1d_6$ relative to the inert internal standard *tert*-butyl alcohol as for **1b** above. The probe of the XL200 NMR spectrometer was preheated to 48.4 °C. Linear least-squares correlation of the natural logarithm of [$1d_6$] vs. time over 3 half-lives gave $k(1d_6) = 7.83 \times 10^{-4}$ s^{-1} with a correlation coefficient $r = 0.966$. Since $k(1b) = 31.5 \times 10^{-4}$ s^{-1} at this temperature (see Table II), the isotope effect $k(1b)/k(1d_6) = 4.02$.

Deuterium Incorporation during Fragmentation of 1b. (a) In Acetic Acid-d_1 Solution. A solution (0.4 mL) containing 4-deuterio-1-methoxybenzene (**27**) (8.7 μL, 8.7 mg, 0.080 mmol), **1b** (40 mg, 0.40 mmol), and acetic acid-d_1 was prepared in a 5-mm NMR tube. The solution was mixed thoroughly by shaking the tube and then heated 3 days at 37 °C. The concentration of the product, levulinaldehyde (**2b**), from decomposition of **1b** was determined by ^1H NMR (60 MHz) spectroscopy relative to the internal standard **27** by using the ratios of integrated areas of the peaks at δ 9.62 (s, 1 H) and 7.27 (br d, 2 H), 6.90 (dd, 2 H) which corresponded to resonances for **2b** and **27**, respectively. Next, the concentration of deuterated levulinaldehyde was determined relative to the internal standard **27** by ^2H NMR (15.36 MHz) spectroscopy. The ratios of the integrated areas of the ^1H-decoupled ^2H NMR signals[33] at δ 2.46 (s, 1 ^2H), 1.89 (s, 1 ^2H), and 6.67 (s, 1 ^2H) which corresponded to the methylene resonances of deuterated levulinaldehyde, methyl resonance of deuterated levulinaldehyde, and **27** were used to calculate the amount of deuterium incorporation in **2b**. The concentration of deuterium found in the methyl and methylene positions of deuterated levulinaldehyde were divided by the total concentration of levulinaldehyde (which was determined from the aldehydic protium resonance) and multiplied by 100 to give the percent deuterium incorporation in each position (Table V).

(b) In Deuterium Oxide Solution. D_2O (0.4 mL) was added to a 5-mm NMR tube containing 40 mg (0.40 mmol) of **1b**. The solution was mixed thoroughly by shaking the tube and then heated at 37 °C in a thermostated oil bath for 1 day. The contents of the NMR tube was poured into a separatory funnel, saturated with NaCl, and extracted with ether (8 × 8 mL). The combined ether extracts were dried over anhydrous magnesium sulfate, and the ether was removed by rotary evaporation (20 mm, 20 °C). The residue was dissolved in 0.3 mL of CCl_4 solution containing 2,3,4,5,6-pentadeuterio-1-methoxybenzene (**26**) (8.9 μL, 9.7 mg, 0.090 mmol) and transferred to a 5-mm NMR tube.

The concentration of the products, levulinaldehyde and 3-hydroxycyclopentanone (**5h**), from decomposition of **1b** was determined relative to the internal standard **26** by ^1H NMR (60 MHz) spectroscopy by using ratios of integrated areas of the peaks at δ 9.72 (s, 1 H), 4.52 (m, 1 H), and 3.74 (s, 3 H) for the two products (levulinaldehyde and **5h**) and **26** (0.30 M), respectively. Next the concentration of deuterated levulinaldehyde was determined relative to the internal standard **26** by ^2H NMR (15.36 MHz) spectroscopy. The ratios of the integrated areas of the ^1H-decoupled ^2H NMR signals[33] at 2.72 (s, 1 ^2H), 2.18 (s, 1 ^2H) and 7.27 (s, 2 ^2H), 6.90 (s, 3 ^2H) which correspond to the methylene resonance of deuterated levulinaldehyde, methyl resonance of deuterated levulinaldehyde, and **26** were used to calculate the amount of deuterium incorporation in the levulinaldehyde product. The concentration of deuterium found in the methyl and methylene positions of deuterated levulinaldehyde were divided by the total concentration of levulinaldehyde (which was determined from the aldehydic protium resonance) and multiplied by 100 to give the percent deuterium incorporation in each position (Table V).

Hydrogen–Deuterium Exchange of Levulinaldehyde (2b). (a) In Deuterium Oxide Solution. A solution (0.40 mL) containing pure levulinaldehyde (**2b**) (40 mg, 0.40 mmol) in D_2O was prepared in a 5-mm NMR tube. The tube was mixed thoroughly by shaking and the ^1H NMR (60 MHz) spectrum was immediately recorded. The ratio (1:1) of hydrated to nonhydrated **2b** was determined by using the ratios of integrated areas of the peaks at δ 5.42 (t, 1 H, $J = 6$ Hz) and 10.08 (s, 1 H) for hydrated and nonhydrated **2b**, respectively. Thereafter, the NMR tube was heated at 37.0 °C in a thermostated oil bath for 2 days. The contents of the NMR tube were then poured into a separatory funnel, saturated with NaCl, and extracted with ether (8 × 8 mL). The combined ether extracts were dried over anhydrous magnesium sulfate, and the ether was removed by rotary evaporation (20 mm, 20 °C). The residue was dissolved in 0.3 mL of CCl_4 solution containing 4-deuterio-1-methoxybenzene (**27**) (6.5 μL, 6.5 mg, 0.059 mmol) and transferred to a 5-mm NMR tube.

The concentration of levulinaldehyde (**2b**) relative to the internal standard **27** was determined by ^1H NMR (60 MHz) spectroscopy by using the ratios of integral areas of the peaks at δ 9.72 (s, 1 H), 2.26 (s, 4 H), 1.97 (s, 3 H), and 3.74 (s, 3 H) which corresponded to signals for the aldehydic, methylene, methyl resonances of **2b** and the resonance of the internal standard **27**, respectively. Next, the amount of deuterium incorporation in **2b** was determined relative to the internal standard **27** by ^2H NMR (15.36 MHz) spectroscopy. The ratios of the integrated areas of the ^1H-decoupled ^2H NMR signals at δ 2.72 (s, 1 ^2H), 2.18 (s, 1 ^2H), and 6.96 (s, 1 ^2H) which corresponded to signals for the methylene and methyl resonances of deuterated levulinaldehyde[33] and the resonance of the internal standard **27** were used to calculate the amount of deuterium incorporated in **2b**. The concentration of deuterium found in the methyl and methylene positions of deuterated levulinaldehyde was divided by the total concentration of levulinaldehyde (which was determined from the aldehydic protium resonance) and multiplied by 100 to give the percent deuterium incorporation in each position (Table V).

(b) In Acetic Acid-d_1 Solution. A solution (0.40 mL) containing pure levulinaldehyde (**2b**) (50 mg, 0.50 mmol), 4-deuterio-1-methoxybenzene (**27**) (43 μL, 43 mg, 0.40 mmol), and acetic acid-d_1 was prepared in a 5-mm NMR tube. The tube was mixed thoroughly by shaking and the ^1H NMR (60 MHz) spectrum was immediately recorded. The concentration of levulinaldehyde (**2b**) relative to the internal standard **27** was determined by ^1H NMR (60 MHz) spectroscopy by using the ratios of integral areas of the peaks at δ 9.62 (s, 1 H) and 3.73 (s, 3 H) which corresponded to resonances for **2b** and **27**, respectively. Next, the amount of deuterium incorporation in **2b** was determined relative to the internal standard **27** by ^2H NMR (15.36 MHz) spectroscopy. The ratios of the integrated areas of the ^1H-decoupled ^2H NMR signals at δ 2.46 (s, 1 ^2H), 1.89 (s, 1 ^2H), and 6.67 (s, 1 ^2H) which corresponded to signals for the methylene, methyl resonance of **2b**, and the resonance of the internal standard **27** were used to calculate the amount of deuterium incorporated in **2b**. The sample was heated in a thermostated oil bath at 37 °C for 2 days, and the concentration of levulinaldehyde and the amount of deuterium incorporated in the methylene and methyl positions was de-

(33) (a) No ^2H NMR signals corresponding to the aldehydic resonance of **2b** (b) or products **5h** or cyclopent-2-en-1-one were detected.

termined by ^1H (60 MHz) and ^2H (15.36 MHz) NMR spectroscopy, respectively. The value obtained for deuterium incorporation was divided by the total concentration of levulinaldehyde (which was determined from the aldehydic protium resonance) and multiplied by 100 to give the percent deuterium incorporation in each position (Table V).

(c) In Dimethyl Sulfoxide Plus Isopropyl Alcohol-d_1 Solution. A solution (0.42 mL) was prepared volumetrically in a 5-mm NMR tube containing 4-deuterio-1-methoxybenzene (**27**) (15 μL, 15 mg, 0.14 mmol, 0.33 M), isopropyl alcohol-d_1 (19 μL, 15 mg, 0.25 mmol, 0.59 M), levulinaldehyde (**2b**) (25 mg, 0.25 mmol, 0.59 M) and dry Me_2SO. The solution was mixed thoroughly by shaking and the concentration of **2b** relative to the internal standard **27** was determined by ^1H NMR (60 MHz) spectroscopy by using the ratios of integrated areas of the peaks at δ 9.62 (s, 1 H) and 7.25 (br d, 2 H), 6.89 (dd, 2 H) for **2b** and **27** (0.33 M), respectively. Next, the amount of deuterium incorporation in **2b** was determined relative to the internal standard **27** by ^2H NMR (15.36 MHz) spectroscopy. The ratios of the integrated areas of the ^1H-decoupled ^2H NMR signals at δ 1.58 (s, 1 ^2H) and 1.14 (s, 1 ^2H) which corresponded to signals for the methylene and methyl resonance of **2b** and δ 5.95 (s, 1 ^2H) for **27** were used to calculate the amount of deuterium incorporated in **2b** (Table V).

The solution was heated at 37 °C in a thermostated oil bath for 60 h. ^1H NMR (60 MHz) and ^2H NMR (15.36 MHz) spectra were recorded periodically, and the amount of deuterium incorporated in the methylene and methyl positions of **2b** was determined and these values divided by the concentration of **2b** (which was determined from the aldehydic protium resonance) and multiplied by 100 to give the percent deuterium incorporated in each position.

Stability of Levulinaldehyde (2b) in Acetic Acid. A solution (2.0 mL) was prepared volumetrically from *p*-dichlorobenzene (35 mg, 0.24 mmol, 0.12 M) and purified acetic acid. Levulinaldehyde (40 mg, 0.40 mmol) (**2b**) was weighed into a 5-mm tube and combined with an aliquot (0.40 mL) of the above solution. The solution was mixed thoroughly by shaking and the concentration of **2b** ([**2b**]) relative to the internal standard, *p*-dichlorobenzene, was determined by ^1H NMR spectroscopy by using ratios of integrated areas of peaks at δ 9.62 (s, 1 H) and 7.22 (s, 4 H) which corresponded to **2b** and *p*-dichlorobenzene, respectively. The NMR tube was heated at 45.0 °C in a thermostated oil bath for 72 h. ^1H NMR spectra were recorded periodically from which [**2b**] was determined relative to the inert internal standard, *p*-dichlorobenzene.

pH Dependence of the Decomposition of 2,3-Dioxabicyclo[2.2.1]heptane (1b) in Aqueous Solution. The reagents used for buffer solutions had the following impurity limits. NaOH: heavy metals as Pb < 6×10^{-4}%, Ni < 1×10^{-3}%, Fe < 1×10^{-4}%, Cu < 1×10^{-3}%. KH_2PO_4: heavy metals as Pb < 1×10^{-3}%, Fe < 2×10^{-3}%. Potassium hydrogen phthalate (KHP): heavy metals as Pb < 5×10^{-4}%, Fe < 5×10^{-4}%. HCl: heavy metals as Pb < 1×10^{-5}%, Fe < 1×10^{-5}%. $Na_2B_2O_7 \cdot 10H_2O$: heavy metals as Pb < 1×10^{-3}%, Fe < 0.5 ppm. Stock reagent solutions were made as follows: A, 0.1 M KHP; B, 0.1 M HCl; C, 0.1 M NaOH; D, 0.1 M KH_2PO_4; E, 0.025 M $Na_2B_4O_7$. Solutions were made and stored in volumetric flasks, decontaminated (by washing with $Na_2Cr_2O_7$, NH_4OH, and Na_2EDTA), and rinsed repeatedly with the low-conductivity H_2O. The stock solutions were mixed to give the following pH values as measured with a pH meter which was calibrated with standard pH 4.00 and 7.00 buffer solutions (pH, buffer mixture): 4.97, 50.0 mL A + 36.6 mL C; 5.95, 50.0 mL D + 5.6 mL C; 7.00, 50.0 mL D + 29.1 mL C; 8.13, 50.0 mL E + 20.5 mL B; 9.00, 50.0 mL E + 4.6 mL B; 9.90, 50.0 mL E + 18.3 mL C.

The buffered aqueous solutions (1 mL) were combined with distilled *tert*-butyl alcohol (10 μL, 7.86 mg, 0.11 mmol) as an internal standard and cooled to 0 °C. 2,3-Dioxabicyclo[2.2.1]heptane (**1b**) (0.020 g, 0.2 mmol) was added to a scrupulously cleaned (with Na_2EDTA) 5 mm o.d. NMR tube. The buffer–internal standard solution (0.2 mL containing 0.02 mmol of *tert*-butyl alcohol standard) was added to the peroxide and the tube shaken to dissolve the peroxide and then immediately placed in the NMR probe which was precooled to 30 °C. The NMR probe was kept at 30 °C throughout the experiment.

After allowing temperature equilibration of the sample (~10 min), the NMR spectrum was recorded and the integral area of the absorption corresponding to the endoperoxide methylene protons (C-5,6) at δ 1.83–1.30 (relative to sodium 2,2-dimethyl-2-silapentane-5-sulfonate) and that of the internal standard, *tert*-butyl alcohol, (δ 1.30–0.90) was recorded at 10.0-min intervals. The decrease of the endoperoxide integral compared with the integral area for the internal standard gave a direct measure of the decomposition rate.

Rearrangements of PGH_2 (1a). (a) In Me_2SO-d_6 Solution. The probe of the XL-100 NMR spectrometer was precooled to 19 °C. Purified PGH_2 (**1a**) (5 mg, 0.014 mmol) stored in dry chloroform-d_1 ($CDCl_3$) in a 5-mm NMR tube at −80 °C was concentrated to dryness at 5 °C by blowing a small steady stream of argon onto the sample. Dry Me_2SO-d_6 (0.35 mL) was added to the NMR tube, and the sample was mixed thoroughly by shaking and then immediately placed into the NMR probe at 19 °C. The first ^1H NMR spectrum (Figure 2A) was generated within a 5-min period in the FT mode while the sample was kept at 19 °C. Thereafter, the probe temperature was raised to 37 °C and ^1H NMR spectra were likewise generated in 5-min periods after the sample had been kept at 37 °C for 20 and 80 min. Both spectra exhibited two new resonances centered at δ 9.57 (d, 1 H) and 9.46 (d, 1 H); however, the H-9 and H-11 resonances of PGH_2 (**1a**) were apparent in the former spectrum but not in the latter spectrum (Figure 2B) which was acquired after the sample had been at 37 °C for 80 min. Therefore, after the sample had been kept at 37 °C for 80 min, the Me_2SO-d_6 was removed by vacuum transfer (20 °C, 0.03 mm) and the residue was taken up in dry $CDCl_3$ and its ^1H NMR (100 MHz) spectrum recorded. The percent yields of levuglandin LGE_2 (**3a**) and lelvuglandin LGD_2 (**2a**) (70–80%) formed from decomposition of PGH_2 (**1a**) were determined by dividing the integral heights for the aldehydic resonances now centered at δ 9.67 (s, 1 H) and 9.53 (s, 1 H) by the integral height corresponding to one hydrogen of the C-20 methyl resonance at δ 0.89 (t, 3 H) and multiplying by 100.

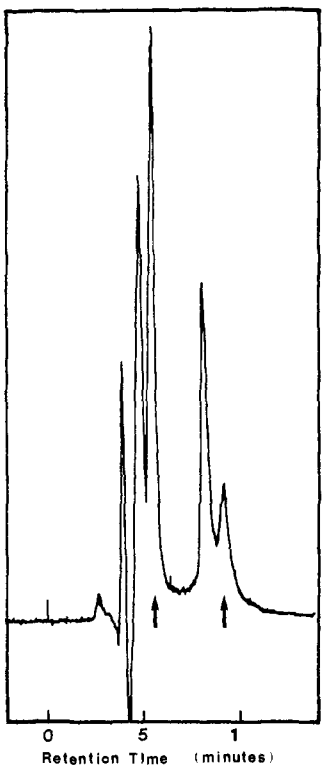


Figure 4. HPLC analysis of products from rearrangement of PGH_2 (**1a**) in Me_2SO at 37 °C.

In a preparative rearrangement, PGH_2 (20 mg) was heated in Me_2SO-d_6 at 37 °C for 85 min. The ^1H NMR spectrum of the product mixture showed aldehyde doublets at δ 9.46 and 9.57 in a ratio of 8:5, respectively. These resonances account for ~70% of the products according to the ratio of their integrated areas vs. that of the C-20 methyl resonance. The Me_2SO-d_6 was removed at 20 °C under reduced pressure (0.01 mm) into a trap cooled to −78 °C. The residue was taken up in dry EtOAc–hexane 60:40 and injected onto a 25 cm × 4.5 mm i.d. Whatman Partisil PX5 10/25 HPLC column with a Waters Associates Series 6000A pump. The components of the mixture were eluted with dry EtOAc–hexane 60:40 at a flow rate of 1.0 mL/min (Figure 4). The ^1H NMR spectrum of the most polar fraction (retention time 9.4 minutes) closely resembled that of the original mixture composed mainly of two aldehydes for which the structures of LGE_2 and LGD_2 are presumed. Upon standing in $CDCl_3$ solution another aldehydic resonance appeared at δ 9.61. Another major, but less polar, HPLC fraction (retention time 5.6 min) also exhibited a new aldehydic resonance at δ 9.61, in this case as the sole aldehydic resonance of the fraction.

(b) In pH 7.9 Buffered D_2O Solution. Prior to the decomposition study of PGH_2 (**1a**) in buffered D_2O a preliminary experiment was performed to determine if aldehydic resonances could be observed in aqueous solvent. Thus, PGH_2 (**1a**) decomposed in Me_2SO-d_6 and then stored in $CDCl_3$ in a 5-mm NMR tube was concentrated to dryness by blowing a small steady stream of argon onto the sample. The residue was taken up in 0.3 mL of a 0.2 M sodium phosphate pH 7.9 buffered D_2O solu-

tion[34] and the ^1H NMR (100 MHz) spectrum recorded. Sharp aldehydic resonances are not observed; however, a broad resonance centered at δ 9.06 was evident.

Purified PGH_2 (**1a**) (9 mg, 0.026 mmol) stored in dry $CDCl_3$ at −80 °C in a 5-mm NMR tube was concentrated to dryness at 5 °C[27] by blowing a small steady stream of argon onto the sample. pH 7.9 buffered D_2O (0.3 mL)[34] was added to the sample, and the solution was mixed thoroughly with shaking and sonication. The sample was placed into the probe of the XL-100 NMR spectrometer at room temperature, and the ^1H NMR spectrum was recorded in the FT mode 10 min after dissolution. The protic impurity from the solvent overlapped with the H-9 and H-11 bridgehead resonances of PGH_2 (**1a**) so monitoring of reaction progress based upon the disappearance of these resonances was not feasible. In addition, no resonances could be seen in the aldehyde region of the spectrum. Therefore, the sample was removed from the probe of the NMR spectrometer and allowed to stand at room temperature for 90 min.[35]

The contents of the NMR tube were transferred to a separatory funnel and acidified to pH 3 with 5% HCl. The aqueous layer was extracted with diethyl ether (5 × 8 mL), and the combined ether extracts were dried over anhydrous Na_2SO_4 (5 °C, 15 h). The ether was removed by rotary evaporation (20 °C, 20 min) and the last traces under high vacuum (20 °C, 0.03 mm). The residue was taken up in dry $CDCl_3$ and the ^1H NMR (100 MHz) spectrum recorded from which resonances centered at δ 9.67 (s, 1 H) and 9.53 (s, 1 H) were evident and were assigned to levuglandin LGE_2 (**3a**) and levuglandin LGD_2 (**2a**). The percent yield of **3a** and **2a** (22%) formed from decomposition of PGH_2 (**1a**) was determined by the cut and weigh method by dividing the weight of the aldehydic resonances at δ 9.67 and 9.53 by one-third the weight of the C-20 methyl resonance centered at δ 0.89 (t, 3 H) and multiplying by 100.

Reaction of Levulinaldehyde (2b) with Glycine Ethyl Ester. A solution (1.0 mL) was prepared volumetrically containing glycine ethyl ester (280 mg, 2.0 mmol, 2.0 M) in D_2O. Another solution (0.26 mL) was prepared volumetrically in a 5-mm NMR tube containing levulinaldehyde (**2b**) (52 mg, 0.52 mmol, 2.0 M) in D_2O. The latter solution was combined with an aliquot (0.25 mL) of the glycine ethyl ester solution. The contents of the NMR tube were mixed thoroughly by shaking and the ^1H NMR (60 MHz) spectrum of the solution was immediately recorded integrating the resonances at δ 10.08 (br t, 1 H), 6.38 (t, 1 H, J = 6 Hz), and 5.42 (t, 1 H, J = 6 Hz) which corresponded to signals for nonhydrated **2b**, an intermediate adduct between **2b** and glycine ethyl ester, and hydrated **2b**, respectively.[36] Next, the solution was slightly basified (pH 10) with dilute NH_4OH and extracted with chloroform (3 × 10 mL). The combined organic layers were dried over anhydrous $MgSO_4$ and chromatographed on a preparative TLC plate (silica gel, 0.5 mm, one development with chloroform (containing 0.75% ethanol)). The pyrrole **24** (R_f 0.29–0.41) (17 mg, 0.104 mmol) was isolated in 20% yield. Spectral data for **24**: ^1H NMR ($CDCl_3$, 60 MHz) δ 6.55 (br t, 1 H), 6.08 (br t, 1 H, J = 3 Hz), 5.91 (br, 1 H), 4.54 (s, 2 H), 4.23 (q, 2 H, J = 7 Hz), 2.18 (br s, 3 H), 1.28 (t, 3 H, J = Hz). High-resolution mass spectrum (70 eV): measured mass (% intensity of base) 167.0638 (100.0), 166.0550 (4.9) ($M^+ - 1$), 139.0362 (1.2) ($M^+ - C_2H_4$), 94.0523 (37.7) ($C_5H_6NCH_2^+$).

Acknowledgment. We thank Professor L. J. Marnett for showing us how to collect and process ram seminal vesicles and teaching us his procedure for bioconversion of arachidonic acid. We thank Professors Marnett, N. A. Porter, and S. R. Wilson for important suggestions. This research was assisted financially by Grant GM-21249 from the Division of General Medical Sciences of the National Institutes of Health.

Registry No. 1a, 42935-17-1; **1b**, 279-35-6; **1d**, 88867-81-6; **1d$_6$**, 91712-40-2; **2a**, 91712-41-3; **2b**, 626-96-0; **2d**, 91712-42-4; **2d$_6$**, 91712-43-5; **3a**, 91712-44-6; **5b**, 26831-63-0; **5d**, 91712-45-7; **5d$_6$**, 91712-46-8; D_2, 7782-39-0.

(34) Sodium phosphate buffer, cf.: Colowick, S. P.; Kaplan, N. O., Eds. "Methods in Enzymology"; Academic Press: New York, 1955; Vol. 1, p 143.

(35) Decomposition of PGH_2 (**1a**) in aqueous media at room temperature is reported[5] to exhibit a $t_{1/2} \sim 20$ min. Therefore, to ensure complete decomposition of PGH_2, we allowed our sample to stand at room temperature for 90 min.

(36) Levulinaldehyde (**2b**) is ca. 50% hydrated in D_2O. However, in this particular experiment, the ratio of non-hydrated **2b** to the intermediate adduct remained at ca. 1:4 for 1 h at 35 °C whereas the resonance at δ 5.42 corresponding to hydrated **2b** was barely detectable.

Regio- and Stereocontrolled Functionalization of Cycloheptadiene Using Organoiron and Organoselenium Chemistry

Anthony J. Pearson,* Sandra L. Kole, and Tapan Ray

Contribution from the Department of Chemistry, Case Western Reserve University, Cleveland, Ohio 44106. *Received January 31, 1984*

Abstract: Whereas the reactions of tricarbonylcycloheptadienyliron salts with nucleophiles generally occur in low yield giving mixtures of products, the corresponding dicarbonyl(triphenylphosphine) and dicarbonyl(triphenyl phosphite) complexes **8** give high yields of single adducts on reaction with a range of nucleophiles. The regioselectivity of nucleophile addition to **8** is strongly dependent on the nucleophile, "soft" nucleophiles attacking C-1, "hard" nucleophiles attacking C-2 of the dienyl ligand. The diene complexes **10** resulting from C-1 addition can be reactivated by hydride abstraction with $Ph_3C^+PF_6^-$, to give dienyl complexes **11**, which undergo a second nucleophile addition regio- and stereospecifically (trans to the $Fe(CO)_2L$ group). Decomplexation is easily accomplished, leading to cycloheptadienylacetic acid derivatives. Phenylselenolactonization of these compounds was accomplished, and the product allylic selenolactones could be subjected to oxidation accompanied by [2,3]-sigmatropic rearrangement of the intermediate selenoxides to give hydroxy lactones. Conformational analysis of substituted (cycloheptadiene)iron complexes and the lactone derivatives is reported.

The use of a transition-metal moiety to control the stereochemistry of consecutive carbon–carbon bond formations on an attached organic ligand offers a unique opportunity for attaining the synthesis of stereodefined organic molecules. We recently demonstrated[1] the efficacy of this method applied to the synthesis of cyclohexenone derivatives, summarized in Scheme I, in which cis stereochemistry is defined during the introduction of substituents at vicinal positions. However, stereocontrol in six- and five-membered rings using a variety of standard organic techniques is well established, so that these ring sizes are inappropriate for demonstrating the utility of the organometallic approach. On the other hand, the cycloheptane ring has received only scant attention[2]

(1) Pearson, A. J.; Ong, C. W. *J. Org. Chem.* **1982**, *47*, 3780.

0002-7863/84/1506-6060$01.50/0