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Nucleophilic Addition of Bisulfite Ion to Prostaglandins E_2 and A_2: Implication in Aqueous Stability

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Abstract □ Evidence is presented to indicate that the bisulfite ion (HSO_3^-) adds across the C-9 carbonyl group of dinoprostone (prostaglandin E_2) and across the $\Delta^{10,11}$-bond of prostaglandin A_2. At room temperature, the apparent equilibrium constant, determined by phase solubility analysis, circular dichroism, UV spectroscopy, and partitioning, for the formation of the bisulfite adduct of dinoprostone is about 7.5 M^{-1} at neutral pH. From this result and a free energy relationship reported in the literature for the thermodynamics of nucleophilic addition to carbonyl groups, it is concluded that the chemical reactivity of the C-9 carbonyl group of dinoprostone is not high enough to improve aqueous stability through reversible one-step nucleophilic reactions. However, from a series of kinetic experiments, it is concluded that the equilibrium is extremely favorable for the formation of the bisulfite adduct of prostaglandin A_2 over pH 4–8 at room temperature. The second-order rate constant for the attack of sulfite ion (SO_3^{2-}) to prostaglandin A_2 is 1.75 $sec^{-1}\ M^{-1}$.

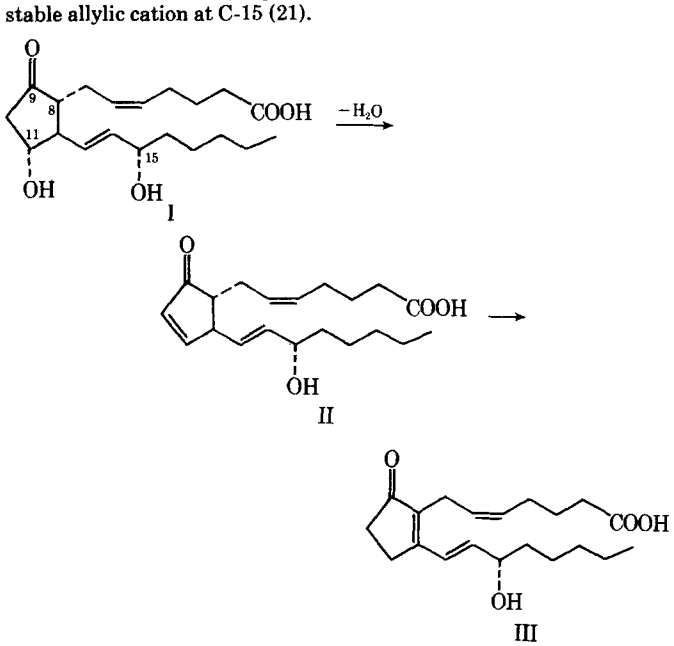
Keyphrases □ Prostaglandins—dinoprostone and prostaglandin A_2, nucleophilic addition of bisulfite ion, effect on aqueous stability □ Dinoprostone—nucleophilic addition of bisulfite ion, effect on aqueous stability □ Nucleophilic addition—bisulfite to dinoprostone and prostaglandin A_2, effect on aqueous stability □ Bisulfite ion—nucleophilic addition to dinoprostone and prostaglandin A_2, effect on aqueous stability □ Stability, aqueous—bisulfite adducts of dinoprostone and prostaglandin A_2

Prostaglandins are analogs of the parent 20-carbon prostanoic acid found in virtually all mammalian cells. After Bergström's pioneering work on the structural characterization and biosynthsis of prostaglandins (1), numerous articles have appeared defining the physiological role and total synthesis of prostaglandins (2). Recently, their general biology (3–5) and chemistry (6–8) were extensively reviewed.

BACKGROUND

Some of the naturally occurring E and F prostaglandins have found wide clinical application in human reproduction (9). In particular, dinoprostone (prostaglandin E_2) (I) has been successfully used for labor induction (10–12). However, the chemical instability of dinoprostone has limited the development of dosage forms, particularly those in aqueous solutions. The instability problem has resulted in a substantial challenge to the pharmaceutical scientist, and this work is part of a fundamental study aimed at exploring the utility of reversible chemical reactions in improving the stability of the E series prostaglandins.

As shown in Scheme I, dinoprostone, being a β-ketol, readily undergoes dehydration to produce prostaglandin A_2 (II) (13–18), which has a different spectrum of biological activity. In aqueous solutions, dinoprostone shows maximum stability at pH 3.5, but the half-life is only about 40 days at 25° (18); prostaglandin A_2, in turn, isomerizes to prostaglandin B_2 (III) in alkaline conditions. In addition to this major degradation reaction, the compound epimerizes at C-8 in the presence of general base (19), presumably because of the high acidity of the proton at C-8 (20). It also epimerizes at C-15 in acid, apparently because of the easy formation of a stable allylic cation at C-15 (21).



Scheme I

Kinetically, dinoprostone appears to undergo dehydration through the E1cB mechanism, with either the formation of enolate at C-9 or the expulsion of OH^- from C-11 as the rate-determining step. Although a thorough kinetic study has to be carried out to identify this proposed mechanism, it is strongly supported by a series of studies (22, 23) on the β-elimination reactions of β-oxy cyclic ketones with various leaving groups of pKa ranging from 5 to 16, including OH^-.

Thermodynamically, the fundamental driving force for the dehydration, which is practically irreversible, appears to be the reduction in free energy content derived from an extended conjugation present in prostaglandin A_2. Therefore, any reversible chemical reactions saturating the carbonyl group at C-9 of dinoprostone should enhance the overall stability through the relationships:

$$\frac{-d(\text{total dinoprostone})}{dt} = k(\text{free dinoprostone}) \qquad \text{(Eq. 1}a\text{)}$$

$$\frac{-d(\text{total dinoprostone})}{dt} = \frac{k}{1+K_{eq}}(\text{total dinoprostone}) \qquad \text{(Eq. 1}b\text{)}$$

where k is the apparent dehydration rate constant of dinoprostone, so long as the attainment of the reaction equilibrium is fast enough that it can be considered as an equilibrium reaction (with an equilibrium constant K_{eq}) prior to the dehydration of dinoprostone and the product of the reversible reaction is chemically stable.

The nucleophilic addition reaction of the bisulfite ion (HSO_3^-) to the carbonyl group at C-9 of dinoprostone was investigated to see if the reaction could be utilized to improve the aqueous stability of dinoprostone. Bisulfite was chosen, among many nucleophilic reagents, because it is relatively nontoxic and hence can be used in pharmaceutical formulations (24) and also because it is an extremely good nucleophile in a thermodynamic as well as a kinetic sense (25, 26). Thus, reactivity of bisulfite with dinoprostone can serve as a measure of the chemical reactivity (*i.e.*, electrophilicity) of the carbonyl group of C-9 of the E prostaglandins in general. The enone system present in prostaglandin A_2 was expected to be very susceptible to nucleophilic addition reactions, as frequently found in many Michael-type 1,4-additions (27). Therefore, it was necessary to study the extent of bisulfite addition to prostaglandin A_2 to draw a complete picture of an aqueous system of dinoprostone containing the nucleophilic reagent.

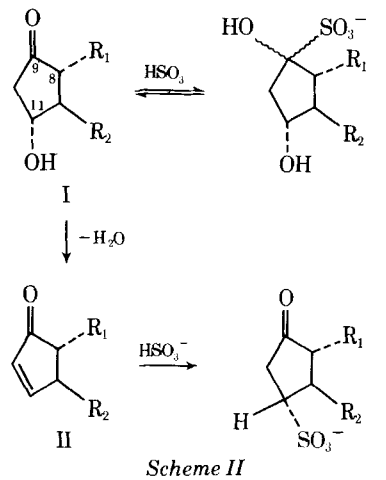
RESULTS AND DISCUSSION

Characterization of Reaction System—It is often difficult to identify chemically a facile equilibrium reaction, especially when the reaction product cannot be easily isolated. Consequently, various physical methods such as UV, IR, and NMR spectroscopic techniques are frequently employed, since in most cases the equilibrium condition is not to be disturbed.

In aqueous solutions, dinoprostone exhibits a negative circular dichroism band near 292 nm with $[\theta] = -1.35 \times 10^4$ due to the $n \rightarrow \pi^*$ transition of the C-9 carbonyl chromophore (5, 28). As shown in Fig. 1, the negative molar rotation was reduced in the presence of the bisulfite ion. At equilibrium, the reduction was a function of the total bisulfite concentration, indicating that the reaction was reversible in nature. If the reaction went to completion, the final spectrum would be independent of the total bisulfite concentration, unless its stoichiometric ratio to dinoprostone concentration was below 1:1.

The ketone carbonyl chromophore of dinoprostone absorbs UV energy very weakly ($\epsilon_{288} \sim 50$), so its absorption band is easily swamped by the bisulfite absorption at the bisulfite concentration level where the reaction can occur to a significant extent. Nevertheless, the absorption peak was significantly reduced upon addition of sodium bisulfite. As in the case of the circular dichroism spectrum, at equilibrium the decrease in absorbance at 288 nm was a function of the total bisulfite concentration. In identifying the reaction, attempts were also made to utilize the carbonyl stretching vibration at 1725 cm^{-1} (5.80 μm) in the IR spectrum of dinoprostone, the C-9 signal at 215.4 ppm (tetramethylsilane) in the ^{13}C-NMR spectrum of dinoprostone, and the quartet at 2.70 ppm due to the equatorial proton at C-10 in the PMR spectrum of dinoprostone. In all cases, supportive but inconclusive data were obtained, mainly because of the small magnitude of the equilibrium constant for the bisulfite adduct formation. From the results obtained from the spectroscopic studies together with the fact that cyclopentanone adds bisulfite (29), it was concluded that the bisulfite ion adds across the C-9 carbonyl group of prostaglandin E_2 in a reversible fashion (Scheme II). At present, the stereochemistry of the addition is not established.

A brief attempt was made to follow the kinetics of bisulfite addition to dinoprostone by monitoring the change in molar rotation at 292 nm,



Scheme II

but no reliable data were obtained because the reaction occurred too rapidly. For example, the rotation at 292 nm of a 1.00-mg/ml solution of dinoprostone containing 0.692 mole of total bisulfite (spectrum C in Fig. 1) was identical after 4 and 20 min of the sample preparation. From this observation and the equilibrium constants measured, it was concluded that the bisulfite addition to dinoprostone is kinetically favorable, even though it is not so favorable thermodynamically.

The high reactivity of the enone system in prostaglandin A_2 is well exemplified by the easy formation of 11-substituted E prostaglandins from the reactions of nucleophiles with the A prostaglandins (28, 30). For instance, hydrogen peroxide adds to C-11 of prostaglandin A_2 to form eventually prostaglandin A_2 10,11-epoxide, an important intermediate in the process of converting prostaglandin A_2 to dinoprostone (31). According to the Sander and Jencks (32) γ-parameter, a measure of thermodynamic nucleophilicity originally derived from a series of reversible nucleophilic addition reactions across a carbonyl group, any nucleophiles of γ-value greater than that of hydrogen peroxide (−0.65) are expected to add to C-11 of prostaglandin A_2, as long as a proper reaction condition is provided. This hypothesis is based on the fact that the thermodynamics governing the nucleophilic addition across a carbonyl group were proved to predict the nucleophilic addition of the reactions across >C=N- (25) and >C=C< (26) bonds. However, no evidence can be found to support that water ($\gamma = -3.58$) adds to prostaglandin A_2 to form dinoprostone. In practice, dehydration of dinoprostone can be considered as an irreversible reaction. On the other hand, the addition of bisulfite ($\gamma = 4.02$)

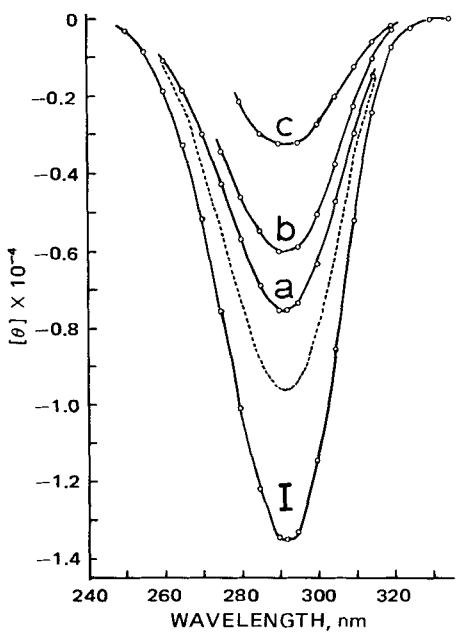


Figure 1—*Circular dichroism spectrum of dinoprostone (I) and its change in the presence of the bisulfite ion at 25°: dinoprostone = 1.00 mg/ml = 2.84 × 10⁻³ M in a phosphate buffer of pH 6.5. Total bisulfite concentrations were 1.042, 0.277, and 0.69 M for a, b, and c, respectively. For comparison, spectrum a in Fig. 2 is shown as a dotted line.*

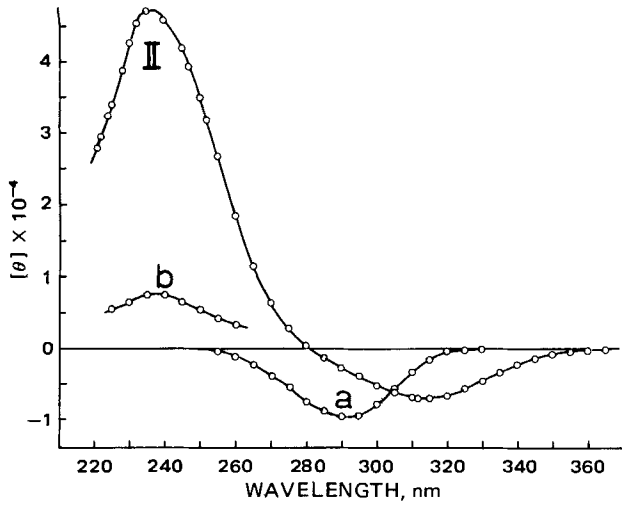


Figure 2—*Circular dichroism spectrum of prostaglandin A_2 (II) and its change in the presence of the bisulfite ion at 25°: prostaglandin A_2 = 0.02 mg/ml = 5.98 × 10^{-5} M in a phosphate buffer of pH 6.5 and I = 0.1 M. Total bisulfite concentrations were 5.38 × 10^{-3} and 2.99 × 10^{-3} M for a and b, respectively.*

to C-11 of prostaglandin A_2 was expected to be very favorable, and even the formation of the 9,11-bis derivative was considered to be possible.

The PMR spectrum of prostaglandin A_2 in deuterated bisulfite buffer of pD = 7.0 (pD = pH read on a meter + 0.4) (33) showed neither the two doublets at 7.6 ppm due to the proton at C-11 nor the two doublets at 6.2 ppm due to the proton at C-10, indicating that bisulfite either adds across the $\Delta^{10,11}$-bond or attacks at C-9 or both. However, the overall spectrum looked similar to that of dinoprostone. Especially at 2.9 ppm, where the equatorial proton at C-11 of dinoprostone was expected to show up as a quartet, poorly resolved but distinctive peaks were still observed, implying the presence of the proton at the carbon alpha to a carbonyl group.

That the C-9 carbonyl group of prostaglandin A_2 is intact in the presence of bisulfite, *i.e.*, that the 9,11-bis derivative is not formed, was also supported by the change in the circular dichroism spectrum of prostaglandin A_2 in the presence of bisulfite. As shown in Fig. 2, prostaglandin A_2 exhibits a strong positive band at 235 nm ($[\theta] = 4.72 \times 10^4$) and a negative band at 312 nm ($[\theta] = -0.69 \times 10^4$) due to the $\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$ transitions of the enone chromophore, respectively. When bisulfite was introduced to a prostaglandin A_2 solution, the spectrum underwent a hypsochromic shift of about 20 nm, and the final spectrum became similar to that of dinoprostone (Fig. 1). This type of spectral change arising from breaking a conjugated system is well known in circular dichroism spectroscopy (34).

The similarity of the final PMR and circular dichroism spectra of prostaglandin A_2 in the presence of bisulfite to those of dinoprostone unambiguously indicates that bisulfite adds across the $\Delta^{10,11}$-bond of prostaglandin A_2 (Scheme II). Because dinoprostone and the bisulfite adduct at C-11 of prostaglandin A_2 are similar to each other in chemical structure, a nearly identical spectroscopic behavior is expected. The possibility of forming the 9,11-bis derivative was ruled out not only by the presence of the C-9 carbonyl group at equilibrium, which was indicated by the PMR study, but also by the fact that only one molecule of bisulfite adds to the enone system in cortisone (35) and to the 1,4-dien-3-one system in dexamethasone (36). Formation of the bis derivative is probably hindered by the electrostatic repulsion between the negative charge on the sulfonate group at C-11 of the monoadduct and the oncoming second molecule of bisulfite.

According to Crabbé (28), various 11α-substituted derivatives of dinoprostone display a more negative Cotton effect with $[\theta] \sim -10{,}000$ at $\lambda_{max} \sim 298$ nm than their corresponding 11β-substituted derivatives. As shown by curve a in Fig. 2, the circular dichroism spectrum of the bisulfite adduct of prostaglandin A_2 is in accord with that of 11α-sulfonate. If this is the case, the stereochemistry involved in the nucleophilic attack at C-11 of prostaglandin A_2 appears to be sterically controlled by the β-side chain at C-12. The PMR study could have given additional information on the stereochemistry of the reaction if an accurate value of ^3J for the coupling of the protons at C-10 and C-11 of the adduct had been available. Such data would give an estimate of the dihedral angle between the deuterium

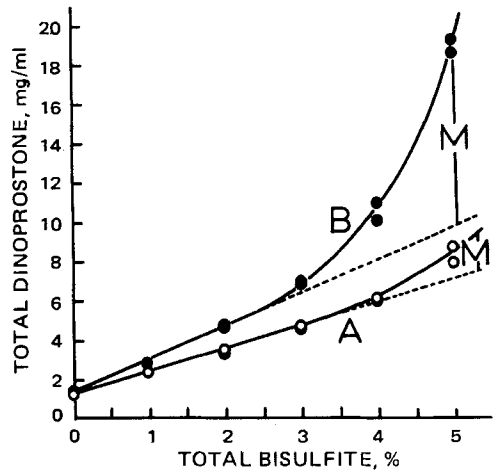


Figure 3—*Aqueous solubility of dinoprostone at 25° as a function of the total bisulfite concentration at pH 3 (A) and 4 (B). The portion showing deviation from linearity (M) indicates the contribution from the micelle formation.*

at C-10 and the sulfonate at C-11. However, as pointed out earlier, the resolution was unfortunately poor at about 3 ppm.

Measurement of Equilibrium Constant for Formation of Bisulfite Adduct of Dinoprostone—Of the many methods commonly employed in determining the equilibrium constant for a reversible reaction or complex formation, phase solubility analysis (37–40), UV and circular dichroism spectroscopic techniques (38, 39, 41), and the partition technique (39, 42) were chosen for the present study. The apparent equilibrium constant is defined as:

$$K = \frac{[ES]}{[E][S]} \qquad \text{(Eq. 2)}$$

where $[ES]$, $[E]$, and $[S]$ are the concentrations of all possible species of the adduct, dinoprostone, and bisulfite, respectively, at equilibrium.

In solubility analysis, if $K[E] \ll 1$, as in the present case ($\sim 10\ M^{-1} \times 2.8 \times 10^{-3}$), then the slope and the intercept of plots of $[E_T]$ *versus* $[S_T]$ become $K[E][S_T]$ and $[E]$, respectively (37, 38), where $[E]$ is the solubility of dinoprostone in the absence of bisulfite, and the subscript T refers to the total concentration of a given species. Therefore, the quantity obtained by dividing the slope by the intercept should be equal to K. However, as shown in Fig. 3, at both pH 3.0 and 4.0, positive deviations from the expected linearity were observed in the plot of $[E_T]$ *versus* $[S_T]$, starting at $[E_T]$ of about 6 mg/ml ($1.7 \times 10^{-3}\ M$). The equilibrium constants estimated from the initial straight lines in Fig. 3 are listed in Table I, together with those obtained by other methods.

The solubility behavior of dinoprostone shown in Fig. 3 can be best explained in terms of mixed micelle formation. The surface tension of a dinoprostone solution (20 mg/ml) at pH 4.0 in 5% bisulfite, measured by the drop-volume technique (43), was about 35 dynes/cm, which is very close to that of a typical surfactant solution (44); the surface tension of a blank solution without dinoprostone was 63 dynes/cm. This result is not surprising, because aqueous prostaglandin solutions are known to be surface active. For example, the dinoprostone-9-dihydro analog, *i.e.*, dinoprost (prostaglandin $F_{2\alpha}$), was reported to form micelles with a

Table I—Equilibrium Constant for the Formation of the Bisulfite Adduct of Dinoprostone at 25°

pH	K, M^{-1}	Method
3.0	10.4	Solubility
4.0	14.4	Solubility
4.9	6.8	UV
6.8	5.0	UV
7.6	2.0	UV
6.5	7.5[a]	Circular dichroism
3.0	5.7	Partition
4.2	6.7	Partition
5.5	8.7	Partition
6.4	10.1	Partition

[a] The most accurate value; see the text for the accuracy level.

critical micelle concentration (CMC) close to 0.02 M (7 mg/ml) (45). In this context, it is interesting that the deviations shown in Fig. 3 start at about 6 mg/ml. At present, the exact chemical composition of the micelle phase is not known.

Equilibrium constants also were calculated from differences in absorbance at 288 nm in the UV spectrum and in molar rotation at 292 nm in the circular dichroism spectrum (Fig. 1) between the buffered solutions of dinoprostone and the same solutions containing various amounts of bisulfite. The values of K calculated by an iterative procedure similar to the one described by Higuchi *et al.* (39) are listed in Table I.

One major assumption made in the partitioning experiment was that the bisulfite adduct of dinoprostone, being an ionic species under the experimental pH range, does not dissolve in the organic layer. Since dinoprostone is highly soluble in ethyl acetate and insoluble in hexane, the 1:1 (volume ratio) mixture of these two solvents was chosen as the organic layer to have an apparent partition coefficient of unity around pH 6. The values of K computed in a conventional manner (42) are collected in Table I. During the partitioning experiment, the apparent pKa of dinoprostone was obtained from a series of blank samples without bisulfite through the relationship:

$$PC' = \frac{(PC)[H^+]}{[H^+] + K_a} \qquad \text{(Eq. 3)}$$

where PC' and PC are the pH-dependent apparent partition coefficient and the intrinsic partition coefficient of neutral species of dinoprostone, respectively. From the data at pH around 5, a pKa value of 4.73 was obtained, which is somewhat lower than the values reported in the literature [5.32 in 85% aqueous ethanol (17), 5.00 (18), and 4.90 for dinoprost (45)].

Data given in Table I are approximately in ±20% error, except the K value obtained from the circular dichroism work, which is in ±5% error. Because of this large experimental error, the pH profile of the K value is not clearly revealed in the present study. As the pH approaches the pKa of the sulfonic acid in the adduct (pKa ~ 1.0), K is expected to decrease since HSO_3^-, a better leaving group than SO_3^{2-}, is formed on the adduct. On the other hand, in alkaline solutions, desulfonation is expected to occur, which is initiated by proton abstraction from the hydroxyl group at C-9 of the adduct. In fact, desulfonation occurring in acidic or alkaline conditions is widely used in purifying cyclic ketones (46).

Because of the low equilibrium constant (~7.5 M^{-1}) for the reaction of the bisulfite ion with dinoprostone, at least a 12.5% (1.2 M) sodium bisulfite solution is required to achieve a mere 10-fold increase in the aqueous stability of dinoprostone when the total concentration of dinoprostone is about 1 mg/ml (2.84×10^{-3} M). Under this condition, not only the toxicity but also the side reaction of the bisulfite ion with other functional groups in dinoprostone becomes troublesome. The side reaction observed is believed to be the substitution of the allylic hydroxyl group at C-15 by the bisulfite ion, since dinoprost, a dinoprostone 9-dihydro analog, also slowly reacts with the bisulfite ion when the latter is in a 100-fold excess of the former. Also, the related allylic system such as the benzylic hydroxyl group in epinephrine is known to undergo substitution with the bisulfite ion (47).

A patent was issued to a claim that the E prostaglandins can be stabilized by incorporation of bisulfite salts (48). Since the systems described in the patent were very rich in alcohol content (50% or sometimes up to 90%), the claimed stability might be the result of the change in the solvent composition rather than the reversible addition of the bisulfite ion to the prostaglandins.

Since the equilibrium constant is only about 7.5 M^{-1}, other nucleophiles known to be *thermodynamically* inferior to bisulfite (25, 32, 49) are not expected to add significantly to dinoprostone in aqueous solutions. Such a consideration of the linear free energy relationship governing the thermodynamics of nucleophilic addition to carbonyl groups appears to indicate that the chemical reactivity of the C-9 carbonyl group of dinoprostone is not high enough to be utilized in improving aqueous stability through reversible one-step nucleophilic reactions.

Kinetics of Bisulfite Addition of Prostaglandin A_2—Even though mathematical manipulation of the rate expression for a reversible reaction is generally more complicated than for an irreversible reaction, it is necessary to treat its kinetics by assuming that the reaction is reversible when it is not known whether the reaction can be considered practically irreversible or not. The reaction:

$$A + S \underset{k_{-1}}{\overset{k_2}{\rightleftharpoons}} AS$$

Scheme III

where A, S, and AS are all possible species of prostaglandin A_2, bisulfite, and the bisulfite adduct of prostaglandin A_2, respectively, can be schematically represented as:

$$A \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} AS$$

Scheme IV

under pseudo-first-order kinetic conditions that can be established in the presence of a large excess of S over A, where $k_1 = k_2(S)$. The rate expression for Schemes III and IV becomes:

$$-\frac{d[A]}{dt} = k_2[A][S] - k_{-1}[AS] = k_1[A] - k_{-1}[AS] \qquad \text{(Eq. 4)}$$

One easy way to determine k_1, k_{-1}, and eventually the second-order rate constant k_2 is to follow the change in absorbance at a given wavelength accompanied by the reaction. Plots of log $(D - D_\infty)$ *versus* time t should result in a straight line where the slope is equal to $-(k_1 + k_{-1})/2.303$, where D and D_∞ are the absorbance at any t and at equilibrium, respectively. If k_{obs} is defined as the sum of k_1 and k_{-1}, then:

$$k_{obs} = k_1 + k_{-1} = k_2[S] + k_{-1} \qquad \text{(Eq. 5)}$$

Under a given reaction condition, k_{obs} is determined at various $[S]$ values, which must be at least 25 times excess over $[A]$ to ensure a pseudo-first-order kinetic condition. From the slope and the intercept of the plot of Eq. 5, k_2 and k_{-1} are obtained. Finally, the equilibrium constant can be obtained from:

$$K = \frac{k_2}{k_{-1}} \qquad \text{(Eq. 6)}$$

The absorption band at λ_{max} 220 nm with $\epsilon_{220} = 1.02 \times 10^4$ of prostaglandin A_2 gradually disappeared in the presence of bisulfite. Figure 4 shows typical plots of log $(D - D_\infty)$ *versus* t with varying concentrations of total bisulfite at pH 6.04. Linearity was observed at least over three $t_{1/2}$ periods. The lowest $[S_T]$ was still about 25 times in excess of the concentration of prostaglandin A_2. From the slopes, k_{obs} values were determined. Isolation of k_2 and k_{-1} from k_{obs} thus obtained is shown in Fig. 5 for all experimental pH values. Values of k_{obs} and k_2 estimated from k_{obs} are listed in Table II.

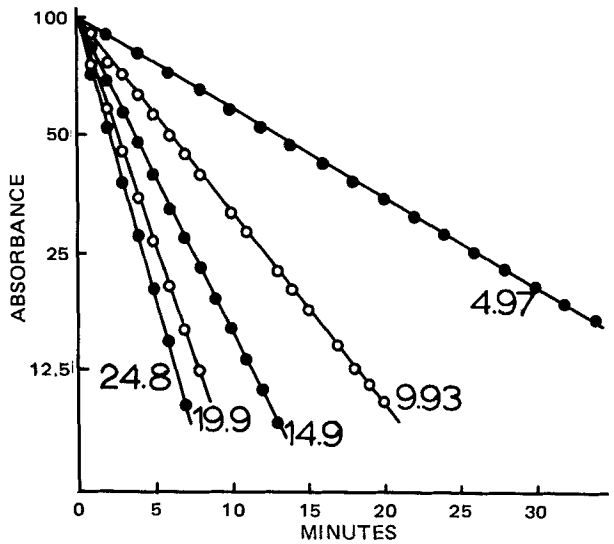


Figure 4—*Semilogarithmic plots against time of absorbance changes that occurred during bisulfite addition to prostaglandin A_2 in a phosphate buffer of pH 6.0 and I = 0.1 M at 25°. Experimental points were normalized to facilitate visual comparisons. Total absorbance changes were at least 0.5 absorbance unit. Prostaglandin A_2 = 2.12 × 10^{-4} M, and total bisulfite concentrations in 10^{-3} M are given on the figure.*

One interesting result indicated by Fig. 5 is the fact that the intercepts k_{-1} are all zero regardless of the pH; *i.e.*, the rate constant for the reverse adduct dissociation is extremely small. This would make the equilibrium constant K for the adduct formation very large over the experimental pH range. From this kinetic study, it is concluded that bisulfite adds across the $\Delta^{10,11}$-bond of prostaglandin A_2 practically in an irreversible fashion over a pH range of 4–8.

The pH dependence of k_2 (*i.e.*, the slope of Fig. 5) is better illustrated in Fig. 6. As pH increases, k_2 also increases, clearly indicating that the kinetically reactive species is SO_3^{2-} and that, under the experimental

Table II—Observed Pseudo-First-Order Rate Constant, k_{obs}, and Second-Order Rate Constant, k_2, for Bisulfite Addition to Prostaglandin A_2 at 24 ± 2°

pH	$[S_T] \times 10^3 M$	λ, nm	$k_{obs} \times 10^2$ min^{-1}	k_2 $min^{-1} M^{-1}$
4.98	11.87	232	1.99	1.43
	17.81	232	2.42	
	23.75	232	4.08	
	23.83	230	3.69	
	29.70	232	4.55	
	35.61	233	5.78	
	39.72	233.5	6.60	
	47.66	235	6.30	
	49.65	233.5	7.22	
5.47	9.93	232.5	4.15	4.05
	19.86	234	7.97	
	29.80	235	12.16	
6.04	4.97	232	5.44	12.00
	9.93	232	11.18	
	14.89	234.5	18.48	
	19.86	236.5	25.96	
	24.82	238	29.00	
6.50	5.93	240	21.7	28.98
	11.87	242	41.2	
	17.80	242	55.9	
	23.74	245	74.25	
	29.67	245	83.2	
7.05	4.97	237	25.7	55.5
	9.93	239	53.8	
	14.89	241	85.4	
7.97	4.97	232.5	46.8	94.0
	9.93	237	92.0	
	14.89	238.5	127.0[a]	

[a] Data not shown in Fig. 5.

conditions, the rate-determining step involves the attack of SO_3^{2-} at C-11. From a mechanistic point of view, protonation of C-10 can occur simultaneously (*i.e.*, 1,2-addition in a true sense) or the sulfite attack can lead to the formation of C-9 enolate (*i.e.*, 1,4-addition). But this point is not the concern of the present study. The reactivity of the $\Delta^{10,11}$-bond of prostaglandin A_2 should not be affected by the ionization of C-1 carboxylic acid (unless there is an unusual proximity field effect between C-1 and C-11). Therefore, when analyzing the pH profile of k_2, it is not necessary to assign a different reactivity to the free acid and to the carboxylate ion. Under this condition, the observed rate expression becomes:

$$\text{rate} = k_2[A][S] = k_2[A][SO_3^{2-}]\left(1 + \frac{[H^+]}{K_a}\right) \qquad \text{(Eq. 7)}$$

where K_a is the ionization constant of HSO_3^- (1.15×10^{-7} at $I = 0.1 M$). Since SO_3^{2-} is the kinetically reactive species, another rate expression is obtained:

$$\text{rate} = k_2^0[A][SO_3^{2-}] \qquad \text{(Eq. 8)}$$

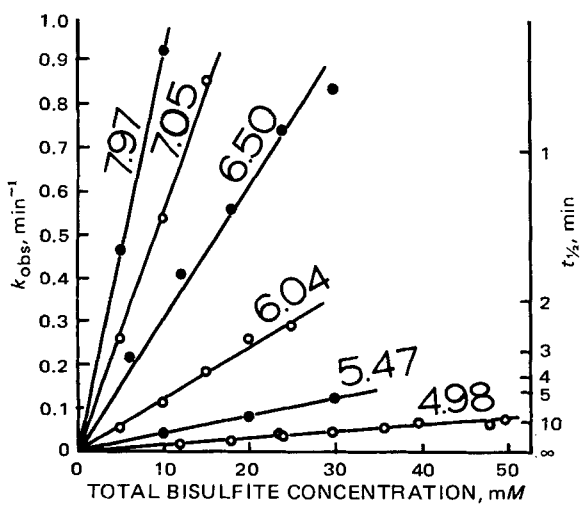


Figure 5—*Relationship of* $k_{obs} = k_2[S] + k_{-1}$ *for bisulfite addition to prostaglandin A_2 at 25° under pseudo-first-order kinetic conditions. The pH values are given on the figure.*

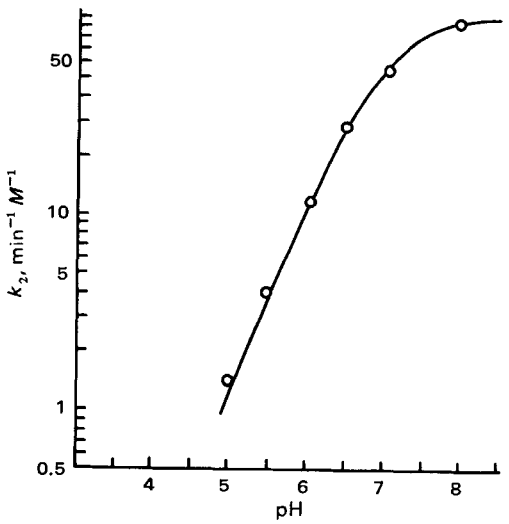


Figure 6—*Dependence on pH of the second-order rate constant,* k_2, *for the formation of the prostaglandin A_2 bisulfite adduct at 25°. The solid curve was theoretically generated using 105 min^{-1} M^{-1} as the value of the pH-independent second-order rate constant,* k_2^0, *for SO_3^{2-} attack on prostaglandin A_2.*

where k_2^0 is the pH-independent second-order rate constant specifically for SO_3^{2-} attack at C-11 of prostaglandin A_2. From Eqs. 7 and 8:

$$k_2 = k_2^0\left(1 + \frac{K_a}{K_a + [H^+]}\right) \qquad \text{(Eq. 9)}$$

By using four values of k_2 at pH > 6, the average k_2^0 of 1.75 $sec^{-1} M^{-1}$ (105 $min^{-1} M^{-1}$) was obtained. The solid curve on Fig. 6 was generated from this value of k_2^0 and Eq. 9. The small but significant (within the experimental accuracy) positive deviation of the experimental data from the theoretical pH profile observed at pH values below 5.5 is attributed to an extra kinetic contribution of HSO_3^- to k_2. The contribution of HSO_3^- is seldom reported as an additional kinetically reactive species; but at a sufficiently low pH range where the fractional concentration of HSO_3^- far exceeds that of SO_3^{2-}, its contribution to the observed rate constant can be clearly detected (25).

EXPERIMENTAL

Reagents and Apparatus—The following chemicals were used without further purification: dinoprostone[1], mol. wt. 352.46, mp 62–64°; prostaglandin A_2[1], mol. wt. 334.46, oily liquid at room temperature; dinoprost[1], mol. wt. 354.46, oily liquid at room temperature; and sodium bisulfite[2] (granular), analytical reagent. All other chemicals were of analytical reagent grade. Buffer systems used were the same as described previously (25). The spectroscopic data were obtained using commercially available instruments[3].

Procedure—The circular dichroism curves of Figs. 1 and 2 are expressed in terms of the molar ellipticity, $[\theta]$, in degrees centimeters[2] decimole^{-1}; $[\theta] = RM/100\,Cl$, where R = rotation (or ellipticity) in degrees, M = molecular weight, C = concentration in grams per milliliter, and l = path length in decimeters. The observed rotation was measured using 0.5-cm cells, except spectrum c. In each case, approximately 30 min passed after a sample solution was prepared when the rotation at 292 nm was measured. Because of the high concentration of bisulfite in Sample c, the noise–signal ratio was very high (dynode voltage was over 0.5 kv). Therefore, spectrum c was obtained using a 0.1-cm cell, and a fivefold increase in sensitivity was employed.

The circular dichroism spectra shown in Fig. 2 were constructed from the observed rotation measured using 1-cm cells for the λ range from 220 to 260 nm and 5-cm cells for the λ range from 250 to 400 nm. About 30 min elapsed after the sample solutions were prepared when the rotation at 285 and 235 was measured for both Samples a and b.

Throughout the phase solubility analysis and the partition experiment, the concentration of dinoprostone was determined by measuring absorbance at 283 nm after dinoprostone had been converted to prosta-

[1] The Upjohn Co.
[2] Mallinckrodt Chemical Co.
[3] Cary 14 spectrophotometer, Perkin-Elmer 621 IR grating spectrophotometer, Varian XL-100-15 spectrometer, and Cary 60 spectropolarimeter.

glandin B_2 in 1 *N* KOH: $\epsilon_{283} = 1.62 \times 10^4$. A quantitative conversion of dinoprostone to prostaglandin B_2 was obtained within 10 min at room temperature.

In phase solubility analysis, excess dinoprostone was added to each of 5-ml buffer solutions containing varying amounts of sodium bisulfite. Seven-milliliter capped[4] vials were used for both solubility and partition experiments. At equilibrium, attained by shaking the samples in a water bath of 25° for 20 hr, the vials were subjected to centrifugation. Undissolved dinoprostone formed an oily droplet at the bottom of the vials, and an aliquot of the supernate was taken out and diluted in 1 *N* KOH. At 25°, less than 2% of total dinoprostone is expected to degrade in 10 hr, since $k_{obs} \sim 10^{-3}$ hr^{-1} under the same conditions (18).

In partition experiments, 3.0 ml of a stock solution of dinoprostone in ethyl acetate–hexane (1:1), which had been saturated with water, and 3.0 ml of a buffer solution in a 7-ml vial were vigorously shaken for 5 hr in a water bath at 25°. At equilibrium, an aliquot of the organic solvent layer was withdrawn; dinoprostone was converted to prostaglandin B_2 after the solvent was evaporated under a nitrogen stream at room temperature. The aliquot from the aqueous layer was directly diluted with 1 *N* KOH.

The equilibrium constant determined by the UV spectrophotometric technique was not as accurate as that determined by circular dichroism spectroscopy, mainly because a wide range of bisulfite concentrations could not be employed. The absorbance of bisulfite solutions is a square (not linear) function of the bisulfite concentration (50).

Because of the UV absorption due to buffer components and bisulfite and other ionic species derived from it (50), the absorbance of λ_{max} of prostaglandin A_2 (220 nm) could not be used in the kinetic study of bisulfite addition to prostaglandin A_2. The results shown in Fig. 4 were, therefore, obtained by following the absorbance change at various wavelengths ranging from 230 to 254 nm (see Table II), using prostaglandin $A_2 = 2.12 \times 10^{-4}$ *M*. The lowest concentration of total bisulfite was about 5×10^{-3} *M*, which satisfies the pseudo-first-order kinetic condition. To protect the bisulfite solution from oxidation, usually a stock solution of about 0.1 *M* was prepared accurately in a deoxygenated buffer solution containing less than 0.5% of methanol as a free radical scavenger. This stock solution was diluted just prior to a kinetic run. To a spectrophotometer cell, 10 μl of stock solution of prostaglandin A_2 in methanol (10 mg/ml) was introduced, and 2.8 ml of a buffer solution containing bisulfite was rapidly added. The absorbance change at a given wavelength was then immediately followed using an identical solution without prostaglandin A_2 as a blank. The ionic strength of the reaction medium varied from 0.1 to about 0.2 *M*, and the temperature of the cell chamber was 24 ± 2°.

REFERENCES

(1) S. Bergström, *Science*, **157**, 382 (1967), and references cited therein.

(2) T. O. Oesterling, W. Morozowich, and T. J. Roseman, *J. Pharm. Sci.*, **61**, 1861 (1972).

(3) J. R. Weeks, *Ann. Rev. Pharmacol.*, **12**, 317 (1972).

(4) J. W. Hinman, *Ann. Rev. Biochem.*, **41**, 161 (1972).

(5) N. H. Andersen and P. W. Ramwell, *Arch. Intern. Med.*, **133**, 30 (1974).

(6) G. L. Bundy, *Ann. Rev. Med. Chem.*, **2**, 157 (1972).

(7) U. Axen, J. E. Pike, and W. P. Schneider, in "Progress in the Total Synthesis of Natural Products," vol. 1, J. W. ApSimon, Ed., Wiley, New York, N.Y., 1973.

(8) N. M. Weinshenker and N. H. Andersen, in "The Prostaglandins," vol. 1, P. W. Ramwell, Ed., Plenum, New York, N.Y., 1973.

(9) "The Prostaglandins: Clinical Applications in Human Reproduction," E. M. Southern, Ed., Futura Publishing, Mount Kisco, N.Y., 1972.

(10) G. A. Murnaghan, H. Lamki, and S. Rashid, *J. Obstet. Gynaecol. Br. Commonw.*, **81**, 141 (1974).

(11) A. W. F. Miller and D. S. Mack, *ibid.*, **81**, 706 (1974).

(12) M. G. Elder and M. Stone, *Prostaglandins*, **6**, 427 (1974).

(13) S. Bergström, in "Prostaglandins," Nobel Symposium 2, S. Bergström and B. Samuelsson, Eds., Interscience, New York, N.Y., 1967, p. 21.

(14) S. M. M. Karim, J. Devlin, and K. Hillier, *Eur. J. Pharmacol.*, **4**, 416 (1968).

(15) N. H. Andersen, *J. Lipid Res.*, **10**, 320 (1969).

(16) T. O. Oesterling, "Abstracts APhA Academy of Pharmaceutical Sciences," Washington, D.C., Apr. 1970, p. 44.

(17) D. C. Monkhouse, L. VanCampen, and A. J. Aguiar, *J. Pharm. Sci.*, **62**, 576 (1973).

(18) G. F. Thompson, J. M. Collins, and L. M. Schmalzried, *ibid.*, **62**, 1738 (1973).

(19) E. G. Daniels, W. C. Krueger, F. P. Kupiecki, J. E. Pike, and W. P. Schneider, *J. Am. Chem. Soc.*, **90**, 5894 (1968).

(20) R. P. Bell and P. W. Smith, *J. Chem. Soc. (B)*, **1966**, 241.

(21) J. E. Pike, F. H. Lincoln, and W. P. Schneider, *J. Org. Chem.*, **34**, 3552 (1969).

(22) L. R. Fedor and W. R. Glave, *J. Am. Chem. Soc.*, **93**, 985 (1971), and references cited therein.

(23) D. J. Hupe, M. C. R. Kendall, G. T. Sinner, and T. A. Spencer, *ibid.*, **95**, 2260 (1973).

(24) L. C. Schroeter, "Sulfur Dioxide," Pergamon, New York, N.Y., 1966, p. 273.

(25) M. J. Cho and I. H. Pitman, *J. Am. Chem. Soc.*, **96**, 1843 (1974), and references cited therein.

(26) I. H. Pitman, M. J. Cho, and G. S. Rork, *ibid.*, **96**, 1840 (1974).

(27) J. March, "Advanced Organic Chemistry; Reactions, Mechanisms, and Structure," McGraw-Hill, New York, N.Y., 1968, p. 568.

(28) P. Crabbé, *Tetrahedron*, **30**, 1979 (1974).

(29) P. Petrenko-Kritschenko and W. Kantscheff, *Chem. Ber.*, **39**, 1452 (1906); through Ref. 24, p. 105.

(30) C. V. Grudzinskas and M. J. Weiss, *Tetrahedron Lett.*, **1973**, 141.

(31) G. L. Bundy, F. H. Lincoln, N. Nelson, J. E. Pike, and W. P. Schneider, *Ann. N.Y. Acad. Sci.*, **180**, 76 (1971).

(32) E. G. Sander and W. P. Jencks, *J. Am. Chem. Soc.*, **90**, 6154 (1968).

(33) T. H. Fife and T. C. Bruice, *J. Phys. Chem.*, **65**, 1079 (1961).

(34) P. Crabbé, "Optical Rotatory Dispersion and Circular Dichroism in Organic Chemistry," Holden-Day, San Francisco, Calif., 1965, pp. 191–243.

(35) A. G. Long and L. J. Wyman (to Glaxo Labs), U.S. pat. 2,908,695 (Oct. 13, 1959); through Ref. 24, p. 111.

(36) G. S. Smith, L. M. Weinstock, F. E. Roberts, Jr., G. S. Brenner, A. M. Hoinowski, B. H. Arison, and A. W. Douglas, *J. Pharm. Sci.*, **61**, 708 (1972).

(37) T. Higuchi and K. A. Connors, in "Advances in Analytical Chemistry and Instrumentation," vol. 4, C. N. Reilley Ed., Interscience, New York, N.Y., 1965, pp. 117–212.

(38) K. A. Connors and J. A. Mollica, Jr., *J. Pharm. Sci.*, **55**, 772 (1966).

(39) T. Higuchi, J. H. Richards, S. S. Davis, A. Kimada, J. P. Hou, M. Nakano, N. I. Nakano, and I. H. Pitman, *ibid.*, **58**, 661 (1969).

(40) W. J. Mader, *Crit. Rev. Anal. Chem.*, **1**, 193 (1970).

(41) J. Meier and T. Higuchi, *J. Pharm. Sci.*, **54**, 1183 (1965).

(42) T. Higuchi and D. A. Zuck, *J. Am. Pharm. Assoc., Sci. Ed.*, **42**, 132 (1953).

(43) E. L. Rowe, *J. Pharm. Sci.*, **61**, 2630 (1972).

(44) B. A. Mulley, in "Advances in Pharmaceutical Sciences," vol. 1, H. S. Bean, A. H. Beckett, and J. E. Carless, Eds., Academic, New York, N.Y., 1964, pp. 86–194.

(45) T. J. Roseman and S. H. Yalkowsky, *J. Pharm. Sci.*, **62**, 1680 (1973).

(46) D. J. Pasto and C. R. Johnson, "Organic Structure Determination," Prentice-Hall, Englewood Cliffs, N.J., 1969, p. 393.

(47) T. Higuchi and L. C. Schroeter, *J. Am. Chem. Soc.*, **82**, 1904 (1960).

(48) D. C. Monkhouse (to Pfizer), U.S. pat. 3,851,052 (Nov. 26, 1974).

(49) J. Hine and R. W. Redding, *J. Org. Chem.*, **35**, 2769 (1970).

(50) D. W. A. Bourne, T. Higuchi, and I. H. Pitman, *J. Pharm. Sci.*, **63**, 865 (1974).

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[4] Lined with Teflon (du Pont).