having IR ($CHCl_3$) 1785, 1775, 1725, and 1700 (sh) cm^{-1}.

Acid Hydrolysis of Acetal 4f. Treatment of 39 mg (0.035 mmol) of acetal **4f** with 2.5 mg of *p*-toluenesulfonic acid monohydrate in 2.5 mL of acetone and 0.25 mL of water according to the procedure described for the hydrolysis of **4a** yielded 29 mg (77%) of aldehyde **7f**[11] having IR ($CHCl_3$) 1780 (broad), 1720 (broad), and 1685 cm^{-1}.

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Kinetics of Epimerization of 15(*R*)-Methylprostaglandin E_2 and of 15(*S*)-Methylprostaglandin E_2 as a Function of pH and Temperature in Aqueous Solution

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Abstract: The kinetics of epimerization of 15(*R*)-methylprostaglandin E_2 (*R*) and 15(*S*)-methylprostaglandin E_2 (*S*) allylic alcohols have been studied in aqueous solution as a function of pH and temperature via high-performance liquid chromatography (HPLC) of their *p*-nitrophenacyl esters. The equilibrium constant was found to be unity within experimental error. The effective rate of epimerization at 37.2 °C was found to be 4.45 $[H^+]$ min^{-1}; the activation energy, E_a, was found to be 20.6 ± 0.4 kcal mol^{-1}. No evidence of reactions competing significantly with epimerization was detected.

Introduction

A number of naturally occurring prostaglandins have been shown to inhibit gastric secretion in animals[1,2] and in man[3-5] and to prevent ulcer formation in rats.[6]

The rapid inactivation of these compounds via oxidation by 15-prostaglandin dehydrogenase limits their therapeutic potential.[7] Incorporation of an alkyl group in place of the C-15 hydrogen in the prostaglandin blocks the action of this enzyme.[8a,b] Synthetic C-15 alkyl substituted prostaglandins have exhibited enhanced potency and duration of action for a number of biological activities. In its antisecretory properties in dogs, 15(*S*)-methylprostaglandin E_2 (*S*) was found to be 30–50 times more potent than prostaglandin E_2 when given intravenously, was active on oral administration, and had a longer duration of activity than prostaglandin E_2.[9] Robert and Yankee have demonstrated that the observed antisecretory activity of the methyl ester of 15(*R*)-methylprostaglandin E_2 (*R*) given orally results from the acid-promoted conversion of this compound to the 15(*S*) epimer.[10] The former compound has no antisecretory activity when administered intravenously.

Robert's and Yankee's observation suggests that the inactive methyl ester of *R* serves as a pro-drug for delivering the epimer active as an antisecretory to its site of action, the gastric mucosa, and only when needed, during the overproduction of gastric acid and pepsin. This delivery system should minimize the side effects associated with the *S* epimer; the most serious of these is the ability to stimulate smooth muscle, particularly the uterus. Karim has demonstrated that *R* is only one-tenth as potent as *S* in its uterine stimulating ability.[11]

The free acid *R* has similar properties to its methyl ester in its ability to inhibit gastric acid secretion and promote the healing of ulcers.[12] The crystallinity of the acid makes this compound easier to formulate as a drug for oral administration than the methyl ester, a viscous oil. The efficacious use of 15(*R*)-methylprostaglandin E_2 clearly is dependent upon its rate of conversion to the active epimer. We report here on the kinetics of this epimerization as a function of temperature and hydrogen ion concentration.

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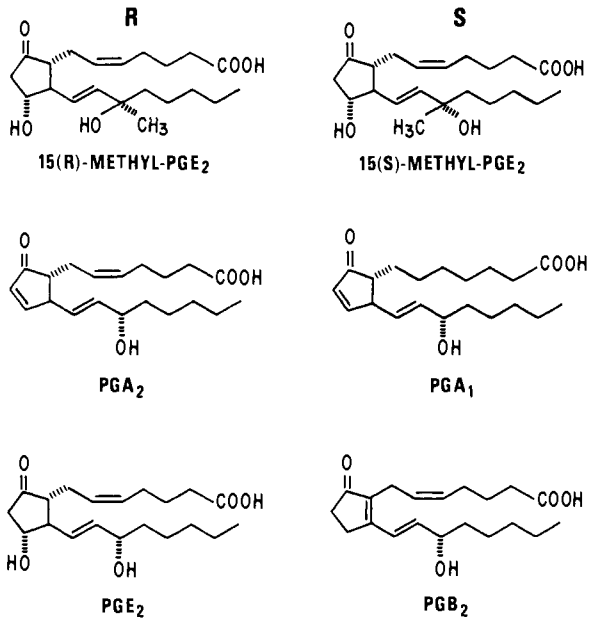


Figure 1. Structures of prostaglandins.

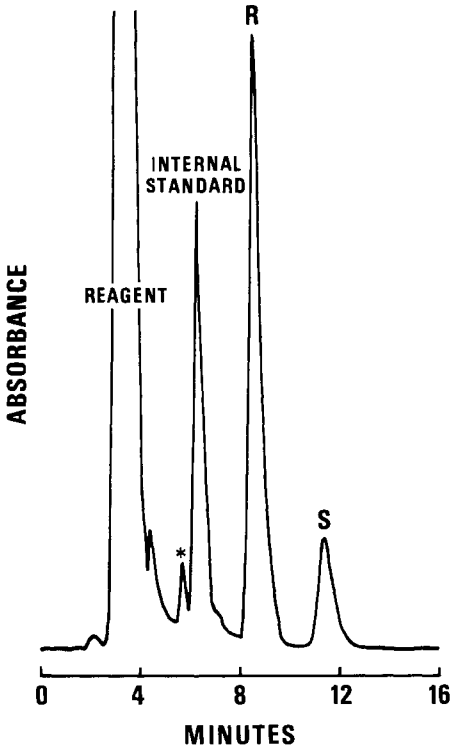


Figure 2. Chromatogram typical of those obtained for each kinetic point. The sample used here was the time = 140 min in kinetic run 4. The asterisk indicates an impurity in the internal standard.

Experimental Section

Chemicals. All prostaglandins and the steroid used as an internal standard were obtained from the research laboratories of The Upjohn Co. Solvents were Burdick-Jackson distilled in glass. Other chemicals were purchased from commercial sources and used without further purification.

Equipment. All chromatography was performed on a Varian 8500 dual pump liquid chromatograph with a Model 635 Variscan UV detector. Detection was at 254 nm. A 25-cm silver ion loaded microparticulate cation exchange resin column was used throughout. The preparation of this column from a prepacked 10-μm Partisil SCX (Reeve Angel) column has been previously described.[13] The column was thermostated at 26 ± 0.1 °C using a Haake Model FJ circulating, constant-temperature bath and a homemade stainless steel column jacket. A 10-μL Precision Sampling Pressure-Lok syringe was used for sample injection.

Samples for kinetic runs were thermostated in a water bath with the temperature controlled to ±0.01 °C with a Haake Model E52 temperature controller calibrated with an NBS thermometer.

Procedure. For each kinetic run, a stock solution was prepared by dissolving an accurately weighed sample of prostaglandin in 8.00 mL of water. To ensure complete dissolution, this sample was shaken overnight on a Burrell wrist-action shaker. A 2.00-mL aliquot of the stock solution was pipetted into 15.00 mL of the appropriate buffer preequilibrated to the desired temperature. This sample was sealed, vigorously agitated, and returned immediately to the water bath. At predetermined times, 1.00-mL aliquots were removed from the kinetic sample and transferred to a 60-mL separatory funnel containing the following quenching buffer and extracting media: 4.00 mL of 0.10 M KH_2PO_4 buffer (pH 7.0), 2.00 mL of Aliquat 336 (General Mills) liquid ion exchanger solution (1.40 mg/mL of $CHCl_3$), 3.00 mL of the internal standard solution, and 2.00 mL of $CHCl_3$. The separatory funnel was vigorously agitated and the lower chloroform layer was removed and dried with ca. 2 g of Na_2SO_4. A 5.00-mL aliquot of the dried chloroform solution was transferred to a 5-mL centrifuge tube and evaporated to dryness with a stream of nitrogen. A 3.00-mL aliquot of an acetonitrile solution of 2-bromo-4′-nitroacetophenone (Aldrich) (0.500 mg/mL) and 1.0 μL of a chloroform stock solution of *N,N′*-diisopropylethylamine (10% v/v) was added to the sample with thorough mixing. The samples were allowed to stand overnight at room temperature. Following removal of the acetonitrile with nitrogen, a 1.00-mL chloroform aliquot was used to dissolve the esterified prostaglandins. A 0.50-mL aliquot of aqueous silver nitrate (200 mg/mL) was added to the sample and the tubes were vigorously mixed; a precipitate formed. After centrifugation, the chloroform layer was passed through a 0.2-μ Millipore filter into a clean container. Duplicate 100-μL injections of this final chloroform solution were chromatographed. Samples not chromatographed on the same day were stored in the cold. Approximately ten points plus three infinity values were taken for each kinetic run. Calibration curves for each epimer were prepared in a similar fashion.

Chromatography for detection and measurement of *R* and *S* as their *p*-nitrophenacyl esters was done using the silver ion loaded Partisil column with a mobile phase consisting of a 61/39 mixture of chloroform/acetonitrile (v/v) and a flow rate of 1.0 mL min^{-1}. A number of the derivatized prostaglandin kinetic samples were examined for the presence of the 15-methylprostaglandin A's and B's via HPLC using a mobile phase of 1% acetonitrile in chloroform on the silver ion loaded Partisil SCX column.

The internal standard solution was a 0.023 mg/mL chloroform solution of 17β-hydroxy-17α-methyl-4-androstene-3,11-dione (11-oxo-17α-methyltestosterone). All the kinetic buffer solutions were 0.033 M in Na_2SO_4. The pH 2.35 solution was ca. 0.01 M HCl. The other buffer solutions were 0.01 M KH_2PO_4, adjusted to the proper pH with hydrochloric acid.

Results

Figure 1 contains the structures of prostaglandins discussed in this report.

Extraction Efficiency. The concentration of Aliquat used here yielded an extraction efficiency of 95 ± 3% for the one-pass extraction based on comparison to an unextracted prostaglandin standard; the extraction efficiency was identical for the two epimers. Sodium nitrate, sodium perchlorate, and sodium sulfate were examined for their effect on the extraction efficiency. Both the nitrate and perchlorate reduced the efficiency of the Aliquat extraction of the prostaglandins. Consequently, sodium sulfate was chosen as the salt for maintaining constant ionic strength of 0.1 M in the kinetic runs. The concentrations of the reagents for derivative formation were optimized for completeness of reaction simultaneous with a low chromatographic background from these reagents for the concentrations of prostaglandins used in this study.

A chromatogram typical of that obtained for each kinetic point is shown in Figure 2.

Examination for Competing Reactions. Only at long time

relative to the half-life required to reach equilibrium were there significant amounts of compounds other than the *p*-nitrophenacyl derivatives of *R* and *S*. In the case of the pH 3.00 run, ca. 3, 6, and 8% of 15-methylprostaglandin A_2 were observed at 3.3, 8.0, and 18 half-lives, respectively, along with approximately twice the amount of two unidentified compounds. No 15-methylprostaglandin B_2 was detected. We were unable to resolve the two 15-methylprostaglandin A_2 epimers; the amounts reported are based on the chromatographic peak height of the *p*-nitrophenacyl ester of a standard sample of 15(*R*)-methylprostaglandin A_2. From their chromatographic retention times, the unidentified compounds were judged to be more polar than prostaglandin A_2 and B_2 type derivatives, but less polar than those of the E_2 series. In samples from pH 2.35 kinetic runs, at ~10 $t_{1/2}$'s, approximately 2.2% of the prostaglandin A_2 derivative was observed along with equal amounts of the two unknown compounds.

Material balance also indicated the lack of reactions competing with epimerization. Using calibration curves based on the peak heights of the *R* and *S* derivatives relative to that of the internal standard, the material balance for each kinetic point was calculated. These data are summarized in Table I. The values reported were those obtained for the first 10 half-lives. The percent recovery ranged from 87.2 ± 6.4 to 115.3 ± 6.0 (precision reported as standard deviation).

Calculation of Rate Constants. The data were fitted assuming a simple first-order equilibrium reaction:

$$R \underset{k'}{\overset{k}{\rightleftharpoons}} S \tag{1}$$

Starting with *R*, the rate law for this reaction can then be expressed as follows:[14]

$$\ln\left(\frac{R_0 - R_e}{R_t - R_e}\right) = (k + k')t \tag{2}$$

R_0 = initial [*R*]
R_e = equilibrium [*R*]
R_t = [*R*] at time *t*

The concentration of either epimer is proportional to the chromatographic peak height of the ester derivative of this material. To eliminate sample to sample variation, the peak height, h_R, was normalized with respect to the total concentration of the two epimers seen in each chromatogram:

$$R_t \propto \left(\frac{h_R}{h_R + Bh_S}\right) \tag{3}$$

h_R = peak height of *p*-nitrophenacyl ester of *R*
h_S = peak height of *p*-nitrophenacyl ester of *S*
B = weighting factor

The constant *B* was derived from the chromatographic data from standards run simultaneously with four separate kinetic runs. Duplicate samples of the same weight of each epimer were dissolved in the chloroform internal standard solution. Aliquots of these solutions were derivatized and chromatographed under conditions identical with those of the kinetic samples; four chromatograms per sample were obtained. The peak height of each epimer relative to that of the internal standard was determined: h_R/h_{IS} and h_S/h_{IS} (h_{IS} = peak height of internal standard).

$$B = \frac{h_S/h_{IS}}{h_R/h_{IS}} \tag{4}$$

The values of *B* obtained in this fashion were averaged with that obtained from the ratio of the slopes of the calibration curves determined at the initiation of this study: $B = 1.41 \pm 0.06$.

Plots of $h_R/(h_R + 1.41h_S)$ and $h_S/(h_R + 1.41h_S)$ vs. time showed that these parameters do reach a constant value. Consequently the assumption that the epimerization reaches equilibrium appeared to be validated.

$$K = \frac{[h_R/(h_R + 1.41h_S)]\ \text{equilibrium}}{[h_S/(h_R + 1.41h_S)]\ \text{equilibrium}} = \frac{0.504 \pm 0.004}{0.496 \pm 0.004} = 1.02 \pm 0.02 \tag{5}$$

The equilibrium constant is unity, within experimental error, and, consequently, $k = k'$.

The pseudo-rate constants of epimerization, $2k$, reported in Table II are the slopes of the line obtained from a linear least-squares fit of the following expression as a function of time:

$$\ln\left[\left(\frac{h_R}{h_R + 1.41h_S}\right)_t - \left(\frac{h_R}{h_R + 1.41h_S}\right)_\infty\right] \tag{6}$$

The value of the pseudo-rate constants obtained using a similar expression for h_S were identical, within experimental error, with those reported in Table II. Correlation coefficients of 0.9987 or higher were obtained for all kinetic runs. An Arrhenius plot (ln $2k$ vs. $1/T$ (K)) yielded a straight line with a correlation of 0.9997. The slope of the line yielded an activation energy, E_a, of 20.6 ± 0.4 kcal mol^{-1}.

A linear least-squares plot of $2k$ vs. [H^+] yielded a line passing through the origin with a correlation of 0.9957 and relative standard deviation of the slope of 4.6%. Consequently, the reaction is first order in hydrogen ion. The rate effective constant for epimerization at 37.2 °C equals 4.45 [H^+] min^{-1}.

Table I. Percent Recovery of *R* as Measured by the Sum of *R* and *S* as Their *p*-Nitrophenacyl Esters via HPLC

Kinetic run	Temp, °C	pH	Initial condition	% recovery
1	37.2	2.35	*a*	95.7 ± 9.2
2	37.2	2.35	*b*	115.3 ± 6.0
3	37.2	2.35	*c*	100.6 ± 4.6
4	37.2	3.00	*a*	105.4 ± 3.3
5	37.2	2.70	*a*	94.3 ± 4.1
6	37.2	3.29	*a*	96.4 ± 4.0
7	32.2	2.35	*a*	88.2 ± 6.2
8	27.2	2.35	*a*	92.7 ± 5.1
9	42.2	2.35	*a*	87.2 ± 6.4

[a] 0.100 mg/mL of *R*. [b] 0.101 mg/mL of *S*. [c] 0.500 mg/mL of *R*.

Discussion

The presence of several chiral centers in *R* and *S* yields nearly identical rotations for these two epimers: $(\alpha_D) \simeq -97°$.[15] Consequently, it was not possible to determine the kinetics of epimerization by monitoring the optical activity. We sought an assay capable of discriminating between *R* and *S* as well as resolving these compounds from products of possible competing reactions.

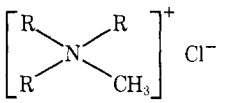
Lustgarten has reported an HPLC separation on a microparticulate silica gel column of *R* and *S* as well as one of the corresponding methyl esters; a refractive index detector was used to monitor these separations.[16a,b] Unfortunately the combination of low solubility of the 15-methylprostaglandin E_2's in aqueous media and low inherent sensitivity of refractive index detectors made this HPLC system unsuitable for the present kinetic study. We have recently reported the HPLC separation of the UV-absorbing *p*-nitrophenacyl esters of a large number of prostaglandins, including *R* and *S*, on a silver

Table II. Rate Constant for Epimerization, $2k$, of 15-Methylprostaglandin E_2 in 0.033 M Na_2SO_4

Kinetic run	Initial condition	Temp, °C	pH	$2k \times 10^3$, min^{-1}	$t_{1/2}$, min	$2k$ min^{-1}/ $[H^+]$
1	*a*	37.2	2.35	20.1 ± 0.1	34.5	4.50
2	*b*	37.2	2.35	19.2 ± 0.2	36.0	4.30
3	*c*	37.2	2.35	18.2 ± 0.2	38.2	4.07
4	*a*	37.2	3.00	3.74 ± 0.03	185.3	3.74
5	*a*	37.2	2.70	7.16 ± 0.07	96.8	3.58
6	*a*	37.2	3.29	1.92 ± 0.02	361.1	3.74
7	*a*	32.2	2.35	11.9 ± 0.1	58.2	2.66
8	*a*	27.2	2.35	6.53 ± 0.3	106.0	1.46
9	*a*	42.2	2.35	34.0 ± 0.2	20.4	7.61

[a] 0.100 mg/mL of *R*. [b] 0.101 mg/mL of *S*. [c] 0.500 mg/mL of R.

ion loaded microparticulate cation exchange resin column.[13] The *p*-nitrophenacyl moiety yielded adequate sensitivity for UV detection for this study. The high resolving power of the silver ion loaded column allowed the separation not only of the ester derivatives of 15-methylprostaglandin E_2's from one another but also from other possible reaction products (see below). Brown and Carpenter had previously used a liquid ion exchanger, Aliquat 336, to extract *R* from an aqueous mixture into chloroform:[17]



R is a mixture of C_8 and C_{10} carbon chains with C_8 predominating. The use of this material permitted the extraction of the two epimers with >95% efficiency under neutral conditions.

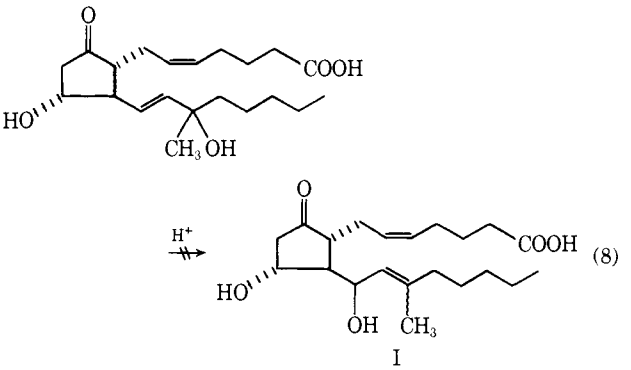
Robert and Yankee showed that the epimerization of *R* and *S* was complete in 0.15 N HCl after 30 min at 37 °C and very slow at pH 3 under the same conditions. The efficacy of the inactive *R* in reducing gastric secretion also indicated that the conversion of this compound to its active epimer was very rapid at high acid concentration. The half-time of epimerization is the time required to yield 75% of the *R* epimer and 25% of the *S* epimer:

$$t_{1/2} = \frac{0.693}{2k[H^+]}\ \text{M min} = \frac{0.693}{4.45[H^+]}\ \text{M min at 37.2 °C} \quad (7)$$

In normal humans, the basal acidity of the fasted stomach is ca. 10^{-2} M (pH 2) in hydrochloric acid.[18] The half-time for epimerization at this acidity is 15 min. In contrast, men with duodenal ulcers have tenfold elevation in stomach acidity (pH 1). The half-time for epimerization under these conditions is 1.5 min. Our kinetic data show, consequently, the efficacy of *R* as a delivery system for the active 15(*S*) epimer in diseased individuals.

Table I indicates some variation greater than random error between kinetic runs of the percentage of starting material recovered as *R* and *S*. These data do show, nonetheless, the absence (<10%) of reactions competing with epimerization. Prostaglandin E readily isomerizes under basic conditions to prostaglandin A and B type compounds. The presence of 15-methylprostaglandin A_2 was detected only at long reaction times and at pH values higher than physiological ones.

In general, the acid-catalyzed rearrangement of a tertiary α-allylic alcohol to a primary or secondary alcohol is quite facile. A well-known example from terpene chemistry is the conversion of linolool to geraniol.[19] Consequently the apparent absence of allylic rearrangement products accompanying epimerization was surprising to us (eq 8). We observed small

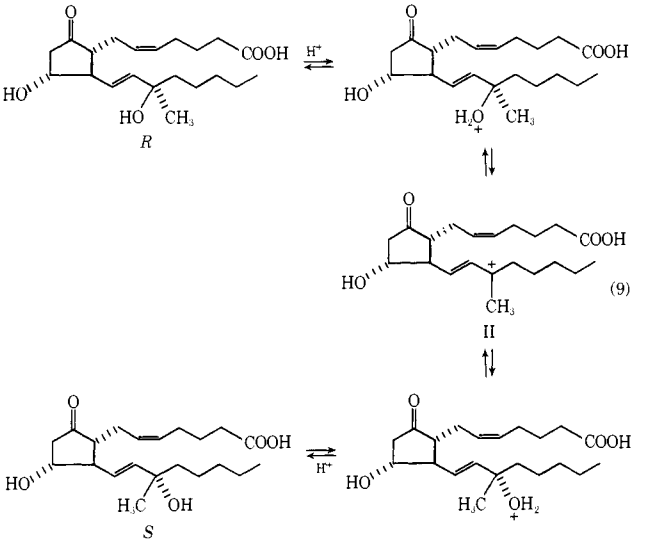


(8)

I

amounts of unidentified compounds less polar than *R* and *S* at long reaction times. Unfortunately authentic samples of I were not available. The polarity of compounds **I**, however, is expected to be greater than those of the starting materials, not less.

The kinetics of the acid-catalyzed rearrangements of allylic alcohols have been investigated extensively in order to elucidate the mechanism of these reactions.[20-24] These studies have established that the conjugate acid of the alcohol can either racemize, in the case of optically active alcohols, or rearrange through a common carbonium ion intermediate. The first-order dependence on hydrogen ion and substrate concentration of the epimerization of the 15-methylprostaglandin E_2 compounds is behavior typical of substituted allylic alcohols[21] (eq 9). The activation energy for the epimerization is also similar



(9)

to that reported for isomerization of α-phenylallyl alcohols[20] and racemization and isomerization of optically active α-methylallyl acetates.[25] The rate of epimerization of *R* and *S* is comparable to that of the isomerization of 1-alkynylallyl

alcohols to the conjugated 3-alkynylallyl compounds.[26] In this latter reaction, rearrangement of the initially formed 1-alkynylallyl carbonium, probably of stability comparable to that of II, is facilitated by formation of a resonance-stabilized secondary carbonium ion. Although there are no reports of allylic rearrangements of prostaglandin E_2 type compounds, Spraggins has observed that treatment of formate esters of 15-epiprostaglandin A_2 with a mixture of methanol and hydrochloric acid yielded 32% of the 13-hydroxy diastereoisomers of prostaglandin A_2.[27] Crystallographic data are not available on prostaglandin A_2. The crystal structure of prostaglandin A_1, however, has been determined.[28] These data indicate that the five-membered ring is nearly planar in prostaglandin A_1, whereas the ring in prostaglandin E_2 is well described as a C-9 half-chair.[29] Examination of molecular models reveals that the nonplanar ring in the E_2 prostaglandins leads to increased steric crowding at C-13 relative to that in prostaglandin A_2. The crowding is further increased by addition of a methyl group at the C-15 position. These steric considerations are probably the major factors in preventing allylic rearrangement during epimerization of *R* and *S*.

Acknowledgment. Helpful discussions with E. W. Yankee and R. K. Lustgarten are gratefully acknowledged.

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Biosynthetic Conversion of Thebaine to Codeinone. Mechanism of Ketone Formation from Enol Ether in Vivo

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Abstract: Biosynthesis of the morphinan alkaloids proceeds by conversion of the enol ether of thebaine to the keto group of neopinone and thence to codeinone. To determine the mechanism of this transformation, [G-^{14}C,6-^{18}O]thebaine was fed to *Papaver somniferum* and the codeine and morphine were isolated. Comparison of the $^{18}O/^{14}C$ ratios in the codeine and morphine isolated with that of the thebaine fed showed that ~34% of the ^{18}O had been retained. Parallel feedings with [G-^{14}C,6-^{18}O]-codeinone demonstrated that the loss was due to nonenzymic exchange. Thus, the mechanism of enol ether cleavage in thebaine is established as cleavage of the 6-*O*-methyl group with retention of the 6-oxygen in the codeinone.

The biosynthetic pathway for the opium alkaloids in *Papaver somniferum* has been shown[2-4] to proceed by the conversion of thebaine (**1**) to codeinone (**3**), probably via neopinone (**2**) and migration of the double bond into conjugation (see Scheme I). Codeinone (**3**) is then reduced to codeine (**4**) which subsequently is 3-O-demethylated to morphine (**5**). Morphine in turn is N-demethylated to normorphine (**6**), and the metabolic fate of the latter has yet to be determined.[5] Thus, in a formal sense, O-demethylations occur at two steps in the pathway: (a) the conversion of thebaine (**1**) to codeinone (**3**) and (b) the conversion of codeine to morphine.

It is reasonable to hypothesize that these two reactions may be catalyzed by different types of enzymes. Since reaction a involves an enol ether and reaction b an aromatic ether, different mechanisms may be involved in their cleavage, as is the case in vitro. Also, morphine and codeine are peculiar to *P. somniferum* (except for very small amounts in *P. setigerum*) while thebaine (**1**) occurs in most other species as well, particularly *P. orientale* and *P. bracteatum*.[3] Oripavine (**7**) also occurs in these two species. These data strongly support the hypothesis that all three species contain an *O*-demethylase which is capable of cleaving the aromatic ether linkage, while *P. somniferum* is unique in that it contains an enzyme system which attacks the enolic ether as well. To establish the nature of the conversion of this enolic ether to ketone is the purpose of the present work.

O-Demethylation of aromatic methyl ethers is a frequent metabolic reaction in mammals, microorganisms, and plants, proceeding by a common oxidative mechanism in all biological systems.[6]

Definitive studies,[7] using $^{18}O_2$ and $H_2{}^{18}O$, showed that oxidative O-demethylation of *p*-methoxyacetanilide by liver

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