^{18}O Isotope Effect in Carbon-13 Nuclear Magnetic Resonance Spectroscopy. 8. Oxygen Exchange of 2,4,6-Trimethylpyrylium Cation[1]

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Abstract: The rate of the oxygen-exchange reaction in an aqueous solution of a heterocyclic oxygen compound, 2,4,6-trimethylpyrylium perchlorate, was quantitated. The ^{18}O isotope effect in ^{13}C NMR spectroscopy affords a direct, continuous, and relatively simple analysis of the reaction and was used to obtain data on the incorporation of ^{18}O from solvent $H_2{}^{18}O$ into the pyrylium salt. Due to the low solubility of this salt in aqueous solutions, 2,4,6-trimethyl[2-^{13}C]pyrylium perchlorate and 2,4,6-trimethyl[2,6-$^{13}C_2$]pyrylium perchlorate were synthesized to facilitate the acquisition of the data on an NTC-200 spectrometer operating at 50.31 MHz. Upon incorporation of ^{18}O into the pyrylium salt, the ^{13}C NMR signal of the directly bonded carbon atom is shifted upfield 0.038 ppm. A ^{13}C isotope effect is also detected in the doubly ^{13}C-enriched pyrylium salt where the ^{13}C NMR signal for ^{13}C-O-^{13}C appears 0.011 ppm downfield from the ^{13}C NMR signal for ^{12}C-O-^{13}C. The rate of the oxygen-exchange reaction was studied as a function of pH (2.0-4.8), buffer concentration, added electrolyte, and temperature. A positive salt effect is observed. The oxygen-exchange reaction is subject to specific hydroxide ion catalysis and to general base catalysis by buffers such as acetate. The ^{18}O-exchange reaction is compared to the other reactions of the pyrylium salt including hydrolysis and ^2H exchange. The apparent energy of activation for the oxygen-exchange reaction at pH 3.1 in the absence of buffer and electrolyte is approximately 25 kcal mol^{-1} while at pH 4.7 in 50 mM sodium acetate, μ = 0.2 M with NaCl, it is 10 kcal mol^{-1}.

Heterocyclic oxygen compounds, e.g., γ-pyrone (4*H*-pyran-4-one), are the structural foundation of many natural products, pesticides, and drugs and are important intermediates in synthetic organic chemistry. Although many properties of heterocyclic oxygen compounds in aqueous solution have been extensively examined, the oxygen-exchange reactions of these compounds have received only superficial experimental scrutiny. These studies include the qualitative analysis of the incorporation of ^{18}O into the heterocycle upon incubation in acidic, neutral, or basic media: 4-pyrone;[2] 4-thio-γ-pyrone;[3] γ-pyrone, kojic acid (5-hydroxy-2-(hydroxymethyl)-γ-pyrone), 5-methoxykojic acid, 6-methylkojic acid, 6-(hydroxymethyl)kojic acid, pyromeconic acid (3-hydroxy-γ-pyrone), maltol (3-hydroxy-2-methyl-γ-pyrone), *allo*-maltol (5-hydroxy-2-methyl-γ-pyrone);[4] 2,4,6-trimethylpyrylium perchlorate;[5] 5-hydroxy-2-methylchromone;[6] chromone.[7] Only in chromone was no oxygen exchange observed. The paucity of data on oxygen-exchange reactions in heterocyclic oxygen compounds and the total lack of quantitative data are largely due to the difficulty in analyzing these reactions by mass spectrometry. Direct mass spectrometric analysis of the heterocycle is often arduous due to limited volatility or to the complicated fragmentation pattern. Degradation of the heterocycle (generally to CO_2), followed by mass spectrometric analysis, is laborious, can introduce undesirable artifacts, and is totally unsatisfactory for heterocycles with more than one exchangeable oxygen atom. Infrared spectroscopy was used to deduce the position of ^{18}O incorporation in one study.[6] The demonstration of the ^{18}O-isotope-induced shift in ^{13}C NMR spectroscopy[8] and its subsequent application for continuous data acquisition in kinetic studies[9] provides an opportunity for the convenient, direct measure of the rate of oxygen exchange in heterocyclic oxygen compounds. In this paper we present for the first time quantitative kinetic data on the rate of oxygen exchange in a ^{13}C-enriched heterocyclic oxygen compound, 2,4,6-trimethyl[2-^{13}C]pyrylium perchlorate and 2,4,6-trimethyl[2,6-$^{13}C_2$]pyrylium perchlorate, utilizing the ^{18}O-isotope effect in ^{13}C NMR spectroscopy.

The chemistry of pyrylium salts has been reviewed in a recent comprehensive monograph.[10] The hydrolysis reactions of several pyrylium salts have been studied in detail,[11,12] as have the reactions of pyrylium salts with amines.[12,13] The latter reaction type is of particular interest in synthetic chemistry as a means for the selective transformation of primary amino groups into other functional groups.[10,14] A quantitative study of the oxygen-exchange reaction in pyrylium salts has been elusive. However, as we demonstrate with 2,4,6-trimethylpyrylium perchlorate, such studies are now quite feasible, and an opportunity is now available to expand the knowledge of the reactions of pyrylium salts. Furthermore, this study extends and complements an examination of the hydrolysis reaction of this specific pyrylium salt by Williams.[11]

Experimental Section

Synthesis of 2,4,6-Trimethyl[2,6-$^{13}C_2$]pyrylium Perchlorate.[15] Sodium [1-^{13}C]acetate (90 atom % excess 1-^{13}C, Stohler) (3 g) was mixed with sodium acetate (1.5 g) and [1-^{13}C]acetyl chloride (60 atom % 1-^{13}C) was synthesized.[16] The pyrylium salt was synthesized by reacting the acid chloride with *tert*-butyl chloride in the presence of aluminum chloride. The yield of the doubly labeled pyrylium salt (60 atom % 2,6-$^{13}C_2$) was 1.5 g (25%).

Synthesis of 2,4,6-Trimethyl[2-^{13}C]pyrylium Perchlorate.[15] Sodium

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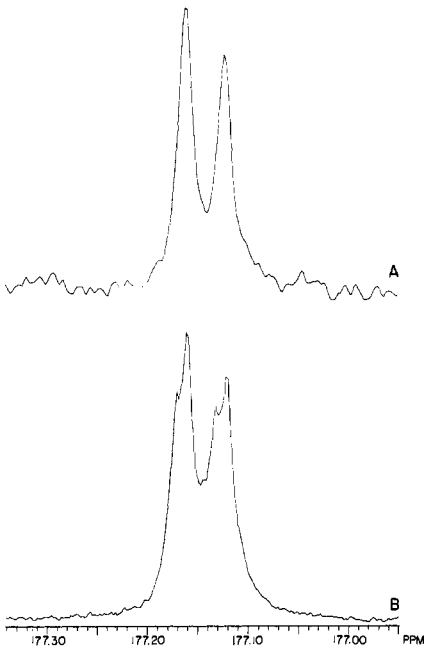


Figure 1. ^{18}O and ^{13}C isotope effects on ^{13}C NMR spectra of labeled 2,4,6-trimethylpyrylium perchlorates. (A) The ^{13}C NMR signal of 2,4,6-trimethyl[2-^{13}C]pyrylium perchlorate dissolved in water is observed at δ 177.16. Upon substitution of ^{18}O for ^{16}O, the ^{13}C NMR signal is shifted upfield 0.038 ppm. A 45% labeling with ^{18}O is illustrated. (B) Superimposed on the ^{18}O-induced spectral shift, additional ^{13}C NMR signals are observed for 2,4,6-trimethyl[2,6-$^{13}C_2$]pyrylium perchlorate. The signals occurring at 0.011 ppm downfield are due to a ^{13}C isotope effect on the ^{13}C NMR signal.

[1-^{13}C]acetate (90 atom % excess 1-^{13}C, Stohler) (1 g) was mixed with sodium acetate (2 g) and [1-^{13}C]acetyl chloride (27 atom % 1-^{13}C) was synthesized.[16] The pyrylium salt was synthesized by reacting the acid chloride with mesityl oxide in the presence of aluminum chloride. The yield of the singly labeled pyrylium salt (27 atom % 2-^{13}C) was 0.5 g (6%) with a melting point of 240 °C.

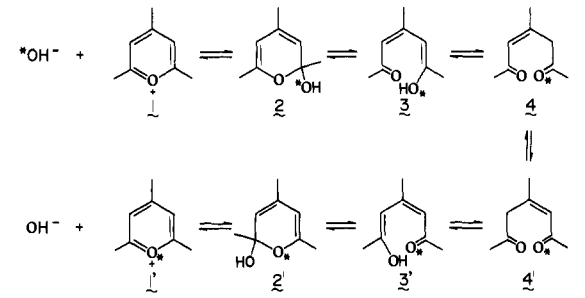
Kinetics of Oxygen Exchange. The solutions for the exchange reactions were prepared by dissolving the salt of the acid used as the buffer and the electrolyte in 40–50% [^{18}O]water (98.5 atom % ^{18}O, normalized, Isotope Labeling Corp.), 20% deuterium oxide (99.75 atom % ^2H, Baker) for instrumental lock, and 30–40% doubly distilled, deionized water; the total volume was 5.0 mL. The pH of the solution was adjusted with 1 M $HClO_4$ and 2 M NaOH. Prior to the addition of the pyrylium salt, the solution was equilibrated at the desired temperature in a circulating water bath for at least 30 min. The NTC-200 spectrometer operating at 50.31 MHz was fitted with a 12-mm probe and equilibrated at the desired temperature for at least 30 min. The reaction was initiated by dissolving the pyrylium salt in the equilibrated solution. A ±400-Hz sweep width (quadrature detection phase), 90° pulse angle, 16K data block, and total acquisition time per spectrum commensurate with the experiment were used. Protons were broad-band decoupled, and a line-broadening factor was applied to the accumulated FID.

The area under each peak was measured, and the relative concentration of each species present was calculated. The pseudo-first-order rate constant for the incorporation of ^{18}O into the pyrylium salt was calculated as previously described for some studies of oxygen exchange at the anomeric carbon atom in carbohydrates.[17]

Results and Discussion

^{18}O- and ^{13}C-Isotope Shifts of the ^{13}C Magnetic Resonance Position. Due to the low solubility of the pyrylium salt in aqueous solution, 2,4,6-trimethyl[2,6-$^{13}C_2$]pyrylium perchlorate was synthesized with 60% ^{13}C enrichment from [1-^{13}C]acetyl chloride and *tert*-butyl chloride,[15] and 2,4,6-trimethyl[2-^{13}C]pyrylium perchlorate was synthesized with 27% ^{13}C enrichment from [1-^{13}C]acetyl chloride and mesityl oxide[15] in order to facilitate the acquisition and evaluation of the data. The chemical shift of the ^{13}C-enriched salt dissolved in water was 177.16 ppm (Figure 1);

Scheme I



this was 3 ppm upfield from the chemical shift observed in CF_3CO_2H/CH_2Cl_2.[18]

The ^{18}O isotope shift of the ^{13}C resonance signal is clearly defined in the spectrum of the [2-^{13}C,^{18}O]pyrylium salt (Figure 1A). Upon substitution of an ^{18}O atom for an ^{16}O atom, the ^{13}C NMR signal of the directly bonded carbon atom in 2,4,6-trimethylpyrylium perchlorate is shifted upfield 0.038 (±0.001) ppm (Figure 1); a 45% incorporation of ^{18}O into the pyrylium salt is illustrated. The magnitude of the isotope-induced shift is that which would be expected for an sp^2-hybridized carbon atom in a carbon–oxygen double bond (carbonyl carbon) conjugated to an aromatic system.[19-21]

In addition to the ^{18}O isotope effect, a ^{13}C isotope effect is also observed in the [2,6-$^{13}C_2$,^{18}O]pyrylium salt (Figure 1B). The ^{13}C NMR signal of the ^{13}C–O–^{13}C pyrylium salt is shifted downfield 0.011 (±0.001) ppm from the ^{13}C NMR signal for the ^{13}C–O–^{12}C pyrylium salt in the unlabeled and in the ^{18}O-labeled species (Figure 1B). In the synthesis of [2,6-$^{13}C_2$]pyrylium salt from [1-^{13}C]acetyl chloride of 60% 1-^{13}C enrichment, the calculated statistical distribution of enrichment is 36% [2,6-$^{13}C_2$], 48% [2-^{13}C], and 16% unenriched; this is the ratio of enriched species observed in Figure 1B.

While heavy-atom isotopic substitution most often results in upfield shifts in the NMR signal of the nucleus being observed, there are scattered reports in the literature of downfield shifts upon heavy-atom isotopic substitution; and the ^{13}C isotope effect (in ^{13}C NMR) observed here is not unprecedented. Deuterium isotope effects, particularly in ^{13}C NMR, account for the majority of reported downfield shifts. Those downfield isotope shifts which were reported through 1982 are included in the comprehensive review on isotope effects in NMR by Hansen.[22] Subsequent to the review, additional downfield isotope shifts have been reported. In ^1H NMR, ^2H substitution results in a downfield induced shift in α-cyclodextrin.[23] In ^{13}C NMR, ^2H substitution was found to induce downfield shifts in the barbaralyl cation,[24] in azulenes,[25] in compounds with conjugated double bonds,[26] in alkyl aromatic and alkyl polynuclear aromatic compounds,[27] and in arylcarbenium ions.[28] In ^{15}N NMR, downfield ^2H isotope shifts in enaminones were observed.[29] One isotope effect of particular interest here was the observation by Jokisaari[30] that ^{13}C at the C-3 (β) position in cyclobutanone shifted the ^{13}C NMR signal of the carbonyl

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Table I. Pseudo-First-Order Rate Constants for the Exchange of the Heterocyclic Oxygen in 2,4,6-Trimethylpyrylium Perchlorate[a,b]

pH	buffer (M)	added electrolyte	temp, °C	10^5k, s^{-1}	$t_{1/2}$, min
2.0	NaH_2PO_4 (0.05)	NaCl	40	<0.6	>2000
3.1	none	none (μ = 0.04 M)	30	0.3	3850
3.1	none	none (μ = 0.04 M)	40	1.1	1044
3.1	none	$NaClO_4$ (μ = 0.14 M)	40	1.2	944
3.2	NaH_2PO_4 (0.05)	none (μ = 0.1 M)	40	2.8	415
3.2	NaH_2PO_4 (0.05)	NaCl	40	4.4	265
4.0	NaAc (0.06)	$NaClO_4$	40	7.9	145
4.0	NaAc (0.25)	$NaClO_4$ (μ = 0.4 M)	40	7.3	158
4.3	NaAc (0.05)	$NaClO_4$	40	16	72
4.6	NaAc (0.05)	$NaClO_4$	40	19.5	59
4.6	NaAc (0.11)	$NaClO_4$	40	18	65
4.6	NaAc (0.21)	$NaClO_4$ (μ = 0.3 M)	40	15	76
4.7	NaAc (0.05)	NaCl	40	9.9	115
4.7	NaAc (0.05)	$NaClO_4$	40	27	43
4.7	NaAc (0.05)	NaCl	30	6.0	192
4.7	NaAc (0.05)	NaCl (μ = 1.1 M)	30	10	111
4.7	NaAc (0.10)	NaCl	20	6.1	190
4.7	NaAc (0.05)	NaCl	20	3.0	388
		(calculated from data at higher temperature)			
4.8	NaAc (0.05)	$NaClO_4$	40	48	24
4.8	NaAc (0.11)	$NaClO_4$	40	86	13

[a] The total ionic strength (μ) is 0.2 M unless otherwise stated and includes the contribution from the pyrylium salt (0.04 M). [b] 20% D_2O.

carbon downfield 0.012 ppm. The magnitudes of the downfield β-^{13}C isotope shift are similar in cyclobutanone (0.012 ppm) and in the pyrylium salt (0.011 ppm).

Oxygen-Exchange Kinetics by NMR. The original study of the heterocyclic oxygen-exchange reaction in 2,4,6-trimethylpyrylium perchlorate was a semiquantitative examination.[5] The pyrylium salt, labeled with 3.76% ^{18}O, was incubated for 30 min at 100 °C in aqueous solutions of sodium acetate/hydrochloric acid or citric acid/sodium phosphate, and the extent of oxygen exchange was measured as a function of pH; the ^{18}O content of the salt was analyzed by mass spectrometric measurement of the ^{18}O in CO_2 obtained upon pyrolysis of the isolated salt. The extent of oxygen exchange over the fixed time interval increased as the pH was raised from 0.65 to 4.00. A mechanism (Scheme I) for the exchange reaction involving hydroxide ion catalysis for formation of the intermediate ketohydroxy tautomer (**3**) and diketo tautomer (**4**) of the pseudobase (**2**)[10,31] was proposed to account for the pH dependence. However, no further studies were undertaken to quantitate the rate of this exchange reaction or to evaluate possible mechanisms such as general base-catalyzed exchange or nucleophilic attack with subsequent general acid-catalyzed exchange. We have re-examined this oxygen exchange reaction using the ^{18}O isotope effect in ^{13}C NMR to illustrate that quantitative data on the rate of oxygen exchange in an oxygen heterocycle may be acquired in a direct, continuous, and relatively simple analysis.

A typical oxygen-exchange reaction for 2,4,6-trimethylpyrylium perchlorate is illustrated in Figure 2. 2,4,6-Trimethyl[2-^{13}C]-pyrylium perchlorate (1.7×10^{-4} mol) was dissolved in a solution of sodium acetate (50 mM), μ = 0.2 M with sodium chloride, pH 4.7, 50% [^{18}O]water/20% deuterium oxide. The solution was incubated at 30 °C, and the incorporation of ^{18}O from the solvent into the pyrylium salt was followed to equilibrium with use of an NTC-200 spectrometer operating at 50.31 MHz.[32] During this period, the initial light blue to colorless solution of the pyrylium salt takes on a yellow color. The ^{13}C NMR signal of the enriched carbon atom is shown at progressive times during the exchange reaction, and the incorporation of the ^{18}O can be followed easily; 3 half-lives of the reaction are illustrated (Figure 2). Data for kinetic plots were obtained by deconvoluting the spectra by using the Nicolet curve resolving program, or by direct peak-height measurements. The pseudo-first-order rate constants for the exchange reaction as calculated from both types of spectral

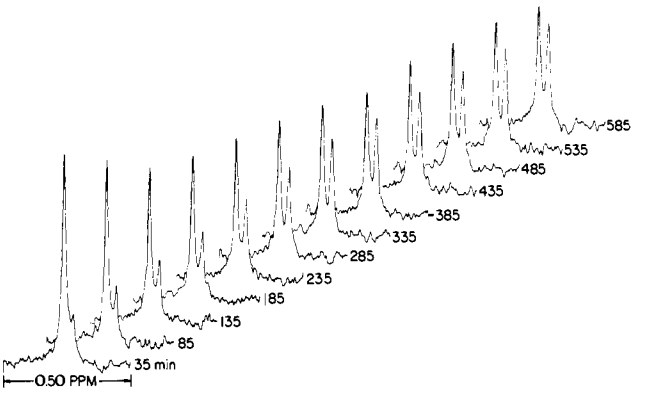


Figure 2. Oxygen exchange of 2,4,6-trimethyl[2-^{13}C]pyrylium perchlorate by NMR spectroscopy. The pyrylium salt (1.7×10^{-4} mol) was dissolved in 50 mM sodium acetate, pH 4.7, μ = 0.2 M with NaCl, 50% [^{18}O]water, 20% deuterium oxide (total volume 5.0 mL) equilibrated at 30 °C. The incorporation of ^{18}O from water into the heterocyclic oxygen position was followed by ^{13}C NMR spectroscopy with use of an NTC-200 spectrometer operating at 50.31 MHz. The presence of ^{18}O induces a 0.038 ppm upfield shift in the ^{13}C NMR signal of the directly bonded ^{13}C atom. The pseudo-first-order rate constant for this reaction is 6.01×10^{-5} s^{-1}, and the 12 spectra illustrate the ^{18}O-exchange reaction for 3 half-lives.

analyses were identical within experimental error. Table I summarizes the experimental conditions of the reactions and the resulting pseudo-first-order rate constants for the oxygen-exchange reactions. The pH was not corrected for the presence of the 20% deuterium oxide, and the total ionic strength includes the contribution of the pyrylium salt. On the basis of a linear least-squares analysis, the standard deviation in k is ±5%.

Previous qualitative observations that the extent of oxygen exchange in pyrylium salts is a function of pH are verified here by the quantitative evaluation of the rates of the reaction. At 40 °C, and under comparable conditions, the rate of oxygen exchange increases as the pH increases from 2.0 to 4.8. Attempts were made to measure the rate of oxygen exchange at pH 5.0 at 40 °C (and at 25 °C); however, no ^{13}C NMR signal for the pyrylium salt was observed; the ^{13}C NMR signal for the pyrylium salt was detected upon acidification of the solution to pH 4.0. In addition, we find

that the rate of this reaction is significantly dependent on added buffer and electrolyte. The energy of activation (E_a) for the reaction at pH 3.1 with no added buffer or electrolyte is approximately 25 kcal mol^{-1}, and the E_a at pH 4.7 with 50 mM sodium acetate and μ = 0.2 M with added sodium chloride is 10 kcal mol^{-1}.

In the absence of added buffers, the pH of an aqueous solution of 2,4,6-trimethylpyrylium perchlorate is 3.1; this value is only slightly altered by the presence or absence of added electrolyte. The rate of the oxygen-exchange reaction under these conditions is very slow at 30 °C and 40 °C, with half-lives of approximately 58 and 17 h, respectively. Upon the addition of electrolyte, $NaClO_4$ (0.10 M), the rate of oxygen exchange increased by 10% at 40 °C. This increase, although relatively close to the experimental error, indicates a somewhat unexpected positive salt effect on the exchange reaction. A 2.5-fold increase in the rate is observed upon the addition of sodium phosphate, monobasic (50 mM) as buffer at 40 °C. The effect of added NaH_2PO_4 buffer and electrolyte, NaCl, to μ = 0.2 M is to increase the rate approximately 4-fold at 40 °C.

The effect of buffer concentration on the rate of oxygen exchange was studied at 40 °C and approximately constant ionic strength by utilizing sodium acetate and sodium perchlorate in the pH range 4.0–4.8. At pH 4.0, when the buffer concentration was quadrupled (and the total concentration of added electrolyte concurrently doubles), there is a fractional decrease in the rate of the reaction. Increasing the concentration of the buffer at pH 4.6 by 4-fold, while the total concentration of added electrolyte increases only fractionally, results in small but quantitatively significant decreases in the rate of the reaction. These observations appear to imply either an electrolyte-induced decrease in the activity of the water—as was observed in the carboxyl(oxygen)–water exchange reaction in acetic acid[16]—or a phenomenon involving the acetic acid component of the buffer or the electrolyte in a "salting-out" reaction. However, at pH 4.8 an approximate doubling of the rate is observed upon doubling of the buffer concentration (at constant concentration of added electrolyte), and an analogous effect on the rate is calculated for the reaction at pH 4.7 with sodium chloride as the electrolyte; these reactions are consistent with a general base-catalyzed reaction involving acetate.

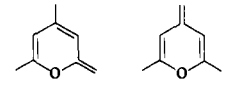
Finally, two additional salt effects were quantitated. At pH 4.7, changing only the electrolyte from sodium chloride to sodium perchlorate results in a 2.7-fold increase in the rate constant. Also increasing the concentration of the electrolyte 10-fold at pH 4.7 gives a corresponding 1.7-fold increase in the rate. These results suggest a significant positive salt effect on the oxygen-exchange reaction. The data are insufficient to evaluate quantitatively the difference between the positive effects exerted by the two salts.

These results are consistent with a general base-catalyzed oxygen exchange reaction of 2,4,6-trimethylpyrylium perchlorate in the presence of a buffer. A specific base-catalyzed or even the slow water-catalyzed exchange reaction may also be observed depending on the pH and presence or absence of buffer components. There is a positive salt effect on the exchange reaction, and ionic strength and dielectric effects are also observed. These data derived from the quantitative evaluation of the rate of oxygen exchange in the pyrylium salt are consistent with the mechanism (Scheme I) proposed on the basis of the qualitative study. It does however seem likely that this minimal scheme should be expanded to include several other possible reactions such as a direct tautomerization between **3** and **3′** and reversible hydration–dehydration equilibria involving the carbonyl compounds **3**, **3′**, **4**, and **4′**.

Similar observations have been reported for the hydrolysis reaction[11] and for the ^2H exchange reaction[33,34] of 2,4,6-trimethylpyrylium perchlorate. Specifically, the rate of the hydrolysis reaction was followed by measuring changes in the UV spectrum of the salt at 25 °C in solutions of acetate, phosphate, and borate buffers (pH 4.84–9.57), μ = 0.1 M. In this pH range the pyrylium salt underwent 100% hydrolysis with virtually no detectable amount of the pyrylium salt in equilibrium with its hydrolysis product(s). General base catalysis, ionic strength, and dielectric effects on the hydrolysis reaction were observed. There was a buffer dependence of the reaction, an absence of acid catalysis, and a significant kinetic deuterium isotope effect. The pH–rate profile showed the existence of an apparent titration (point of inflection approximately pH 6.7). These data indicated that the pseudobase (**2**) decomposed by a pH-independent pathway to yield 4-methyl-3-heptene-2,6-dione or could react to reform the pyrylium salt, although the equilibrium favors the dione to the pseudobase ($K_{eq} > 500$); the apparent titration implied that the pseudobase (**2**) deprotonates to give a reactive species or is formed with simultaneous release of a proton. The hydrolysis product, 4-methyl-3-heptene-2,6-dione, was extracted and characterized by IR, UV, and ^1H NMR spectroscopy; an equilibrium mixture of the cis and trans isomers of the product best accounted for the data. In a review article on heterocyclic pseudobases,[30] Bunting interpreted the experimental evidence to indicate that formation of the pseudobase was general base catalyzed, and thus the decomposition of the pseudobase was general acid catalyzed, at all pH values. Although there are minor differences between the experimental conditions used to study the ^{18}O-exchange reaction and the hydrolysis reaction that may affect specific values measured in the reactions, there is no discrepancy between the ^{18}O-exchange reaction and the hydrolysis reaction. This is as it should be because, in order for oxygen exchange to proceed, a hydrolysis reaction must occur. However, the equilibrium under these conditions favors re-formation of the pyrylium salt rather than formation of the dione.

The ^{13}C NMR spectrum of the [2-^{13}C]pyrylium salt or the [2,6-$^{13}C_2$]pyrylium salt dissolved in aqueous solution shows a single peak at δ 177. However at pH 4.0–4.8 two ^{13}C NMR signals appear at δ 202 and 211, indicative of a conjugated carbonyl group and an isolated carbonyl group, respectively. The areas under these signals increase as the pH is raised from 4.0 to 4.8 and are dependent on the composition of the buffer and salt in the solution. In $H_2{}^{18}O$, these ^{13}C resonances exhibit upfield ^{18}O isotope shifts of 0.042 and 0.052 ppm, which are the isotope shifts of a conjugated carbonyl carbon and an isolated carbonyl group, respectively.[19] These ^{13}C NMR signals have been assigned to the carbonyl groups in the diketo tautomer of the pyrylium salt. Therefore, the ^{18}O exchange reaction may be considered as an extension of the hydrolysis reaction. At pH above ~4.8, the pyrylium salt can no longer be detected, and our failure to be able to follow oxygen exchange at pH 5.0 is understandable. Likewise, in spite of the necessarily different mechanisms, both the oxygen exchange reaction and the ^2H-exchange reaction of the methyl groups in 2,4,6-trimethylpyrylium perchlorate have similar characteristics: the rates of the reactions increase and the E_a values for the exchange reactions decrease as the pH is increased.

The reactions of 2,4,6-trimethylpyrylium perchlorate—hydrolysis, ^{18}O exchange, ^2H exchange, and pyridinium formation—involve direct nucleophilic attack (or its kinetic equivalent) on the pyrylium cation. From the experimental data, the mechanism for the hydrolysis reaction, the ^{18}O-exchange reaction, and the ^2H-exchange reaction is general base catalysis of the formation of the reactive intermediate and general acid catalysis of the re-formation of the pyrylium cation. The reactive intermediate for the three reactions is the pseudobase (**2**) and its ketohydroxy (**3**) and diketo (**4**) tautomers (the latter also being the product of the hydrolysis reaction, 4-methyl-3-heptene-2,6-dione); ^2H exchange also involves a methylenepyran (**5**, **6**) intermediate.[33,34] Under appropriate conditions (e.g., pH 4–4.8),



5 **6**

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(32) Under these experimental conditions, only the oxygen exchange of the pyrylium salt can be observed. Oxygen exchange of acetic acid is much slower and presents no complicating factor.[16]

these three reactions proceed simultaneously at equilibrium. The hydrolysis reaction and ^{18}O-exchange reaction are subject to ionic strength and salt effects. Although the pseudobase tautomer **2** of the 2,4,6-trimethylpyrylium salt appears to be so reactive that it has not yet been identified, pseudobases of other derivatives such as the flavylium ion have been observed.[31,35] The present study provides information about the existence and reactivity of intermediates such as **3** and **4**. Starting with the recently reported compound 4-methyl-4-hydroxy-2,6-heptanedione,[36] a direct synthesis of the diketone hydrolysis product might be achieved by dehydration. Further insight into the details of the reactions of the pyrylium salt, including its formation, should be obtainable.

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Registry No. 2,4,6-Trimethylpyrylium perchlorate, 940-93-2; 2,4,6-trimethyl[2,6-$^{13}C_2$]pyrylium perchlorate, 93111-97-8; 2,4,6-trimethyl[2-^{13}C]pyrylium perchlorate, 93111-99-0; [1-^{13}C]acetyl chloride, 1520-57-6; *tert*-butyl chloride, 507-20-0; aluminum chloride, 7446-70-0; mesityl oxide, 141-79-7; oxygen-18, 14797-71-8.

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Molecular Recognition in Model Crystal Complexes: The Resolution of D and L Amino Acids

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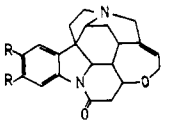
Abstract: The structures of the molecular complexes of *N*-benzoyl-L-alanine + strychnine and *N*-benzoyl-D-alanine + brucine have been determined by X-ray crystallography. The crystal structures of these two complexes provide for the first time some insight into the mechanism of the resolution of racemic mixtures of amino acids. The two methoxy groups on brucine produce a dramatic difference in its packing arrangement compared to that of strychnine. Wedge-shaped brucine molecules form corrugated monolayer sheets which concertina together to form a inclusion complex. In contrast, the strychnine molecules are arranged in bilayers. Despite the very different molecular environments, the L and D enantiomers of benzoylalanine adopt conformations closely related by mirror symmetry.

Separation of racemic mixtures was first achieved by Pasteur in 1853,[1] and the principles he developed are still commonly used today. An optically pure enantiomeric base is added to a racemic mixture of D and L acid. In a large number of cases it is found that one of the diastereomeric salts (base + L acid or base + D acid) is very much less soluble and preferentially crystallizes out of solution. The first amino acids were resolved by Emil Fischer in 1899[2] using the naturally occurring alkaloids strychnine and brucine (I) to separate the racemic *N*-benzoyl derivatives of alanine, glutamic acid, and aspartic acid. Approximately 2000 organic compounds have been reported in the literature[3,11] as being resolvable by the formation of diastereomeric salts, and for compounds with acid functional groups, strychnine and particularly brucine have been widely used as resolving agents. Successful resolutions can only the achieved by a time-consuming trial-and-error procedure in which various resolving agents and solvents are tested. Despite the practical importance of this technique, there is no explanation or rationalization of any of the many experimental results. Apart from work in this laboratory,[7,10] there are only three published crystal structures involving strychnine or brucine: strychnine bromide,[8] strychnine sulfate,[9] and the 1:1 complex brucine + 1-(*O*-bromophenyl)-1-phenyl-2-propynol.[5]



I

strychnine, R = H
brucine, R = OMe

Crystal Structure of Strychnine and *N*-Benzoyl-L-alanine

Crystals of this complex were prepared by using the method described by Pope[4] and were found to be orthorhombic with space group $P2_12_12_1$ and unit cell dimensions a = 10.751 Å, b = 30.366 Å, c = 8.608 Å. All hydrogen atoms were located, and the structure was refined to give a final R = 0.04; full details of the structure determination will be published elsewhere. A view of the unit cell is shown in Figure 1.

The crystal is composed of bilayers of strychnine molecules separated by hydrogen-bonded sheets comprising the carboxyl oxygen atom and the two solvent water molecules. Crystal

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