

SUMMARY

A gas chromatographic method has been developed for the determination of caffeine in plasma. A standard response curve relating signal-height ratios of caffeine to hexobarbital permits a quantitative estimation of the amount of caffeine present. The method is more rapid and sensitive than any previously reported. Plasma levels of caffeine after the ingestion of one cup of coffee have been studied and concentrations down to 0.25 mcg./ml. have been accurately determined.

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Keyphrases

Caffeine in plasma-analysis
 Plasma analysis-caffeine
 GLC-analysis
 Hexobarbital-internal standard

Oxidation of Organic Compounds with Xenon Trioxide I

Stoichiometric and Kinetic Studies Involving Cinnamyl Alcohol, Cinnamaldehyde, and Cinnamic Acid

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The stoichiometry involved in the xenon trioxide oxidation of *cis*- and *trans*-cinnamyl alcohol, *trans*-cinnamaldehyde, and *trans*-cinnamic acid to carbon dioxide and water was determined using an excess of xenon trioxide. The rate of oxidation of *trans*-cinnamaldehyde with xenon trioxide within the concentration range studied, was found to be first order with respect to xenon trioxide and zero order with respect to *trans*-cinnamaldehyde. The reaction rate is pH-dependent and inversely related to the ionic strength of phosphate buffer.

STUDIES INVOLVING the preparation and oxidizing properties of xenon trioxide in aqueous

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solution have been cited in an earlier paper (1), which described the determination and stoichiometry involved in the oxidation of certain primary, secondary, and tertiary aliphatic and aromatic alcohols.

The present investigation concerns the xenon

trioxide oxidation of *cis*- and *trans*-cinnamyl alcohol, *trans*-cinnamaldehyde, and *trans*-cinnamic acid. The stoichiometry of the oxidation of these compounds to carbon dioxide and water by xenon trioxide was determined. In addition, for *trans*-cinnamaldehyde, the following were determined: the reaction order with respect to xenon trioxide and aldehyde, the pH profile for the reaction, and the effect of increasing ionic strength of phosphate buffer on the reaction rate.

EXPERIMENTAL

Apparatus—The pH of reaction mixtures and buffers was determined with a Beckman pH meter, model 76A.

Solutions and Reagents—Highly purified water, which was used for preparing all solutions and dilutions, was obtained by passing the vapors through a column of cupric oxide heated to 800°.

Xenon trioxide solution (0.02 *M* and 0.09 *M*) was prepared by diluting a concentrate (0.2 *M*) with highly purified water. The preparation of the concentrated xenon trioxide solution by hydrolysis of xenon hexafluoride has been described by Appelman and Malm (2).

The following stock solutions of the compounds studied were prepared: 0.0020 *M cis*- and *trans*-cinnamyl alcohol, 0.0020 *M*, 0.0060 *M*, and 0.0080 *M trans*-cinnamaldehyde, and 0.0020 *M trans*-cinnamic acid.

Phosphate buffer solutions used in the kinetic studies were prepared in concentrations of 0.20 *M*, 0.50 *M*, and 0.75 *M* and at pH values ranging from 2 to 6.8 from standard buffer solutions (Fisher Scientific Co., Fair Lawn, N. J.), monobasic potassium phosphate, dibasic potassium phosphate, phosphoric acid, and potassium hydroxide.

Sodium thiosulfate solution (0.0050 *N*) was prepared by diluting a standard 0.020 *N* solution (Fisher Scientific Co., Fair Lawn, N. J.).

Sulfuric acid solution, 10% w/v, was prepared by diluting the concentrated form with highly purified water.

Potassium iodide and starch test solution were obtained from Fisher Scientific Co., Fair Lawn, N. J.

All chemicals and solvents were reagent grade.

Procedure—The stoichiometry involved in the xenon trioxide oxidation of *cis*- and *trans*-cinnamyl alcohol, *trans*-cinnamaldehyde, *trans*-cinnamic acid to carbon dioxide and water was determined by the following procedure, which is a modified version of the one described in a previous paper (1). In each case, exactly 1.0 ml. of 0.02 *M* xenon trioxide, a known excess, was added by micropipet to a series of 50-ml. glass-stoppered conical flasks. Aliquots of the 2.0×10^{-3} *M* substrate stock solution, ranging in volume from 0 to 1,000 μ l., followed by a sufficient quantity of highly purified water to bring the total volume to 10 ml., were then added to the flasks. The flasks were stoppered and allowed to stand at room temperature. Since the time required for complete oxidation depends on the nature of the substrate and its concentration, preliminary studies were run to determine these values. Complete oxidation was noted in 9 days for the alcohol, 12 for the aldehyde,

and 13 for the acid. After the reaction had gone to completion, the excess xenon trioxide was determined by the method of Appelman and Malm (2), which involves acidifying the reaction solution with 4 ml. of diluted sulfuric acid (10% w/v), adding potassium iodide (1 g.), and titrating the triiodide formed with standard sodium thiosulfate (0.020 *N* or 0.005 *N*) from a 10-ml. buret graduated to 0.01 ml. The end point sharpness was increased by addition, as it was approached, of three drops of starch T.S. to serve as an indicator. The amount of xenon trioxide consumed in the oxidation was determined by subtracting the amount found in excess from that found in a flask to which no substrate was added initially (blank). Thus, the millimoles of xenon trioxide reacting were determined from the normality of the thiosulfate solution and the difference in the volumes consumed by the blank and the sample. The millimoles of xenon trioxide consumed in the oxidation was then plotted against the millimoles of substrate taken. The slope and intercept of the plot were determined by regression analysis (1).

The reaction between *trans*-cinnamaldehyde and xenon trioxide was used for the kinetic studies, rather than the alcohol, because of its more favorable rate of oxidation. All experiments were carried out at $30 \pm 0.1^\circ$ employing a large excess of the aldehyde relative to xenon trioxide. The reaction rate was followed by noting the change in xenon trioxide concentration. Xenon trioxide was determined as described previously. The reaction was carried to at least 50% completion. The procedure is described below.

Aliquots of a 6.0×10^{-3} *M trans*-cinnamaldehyde stock solution containing 0.2 *M* phosphate buffer (pH 5.35), ranging in volume from 60–180 ml., were pipetted into a series of 500-ml. glass-stoppered conical flasks. With the exception of the last stock solution, a sufficient quantity of 0.2 *M* phosphate buffer (pH 5.35) was subsequently added to each to bring the volume to 180 ml. The flasks were then covered with glass stoppers and placed in a thermostatically controlled heating bath previously adjusted to $30 \pm 0.1^\circ$. Xenon trioxide (200 μ l., 0.09 *M*) was added to each at zero time. Aliquots (15 or 20 ml.) of the reaction mixture were removed at six time intervals ranging from 0.5 to 9 hr. The reaction was quenched by adding 1 g. of potassium iodide. The quantity of xenon trioxide was determined as previously described.

To determine the reaction order with respect to xenon trioxide the concentration of xenon trioxide remaining in the reaction mixture was plotted against reaction time on semilogarithmic graph paper. The slope, determined by calculating the least-square regression line, was used to obtain the rate constant. The half-life of the reaction was calculated from the rate constant by the expression: $t_{0.5} = 0.693/k$.

To determine the reaction order with respect to *trans*-cinnamaldehyde, the rate constants were determined at various aldehyde concentrations.

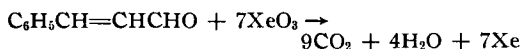
The pH profile was determined at $30 \pm 0.1^\circ$ in 0.2 *M* phosphate buffer at six pH intervals (1.4–6.8). Xenon trioxide solution (200 μ l., 0.09 *M*) was added at zero time to each of six buffered solutions of *trans*-cinnamaldehyde (100 ml., 0.0060 *M*), previously adjusted to the desired pH and temperature. The pH of the reaction mixture was then measured with a pH meter. The reaction was followed to at least 50%

completion. The rate constants of the reactions, determined as described previously, were then plotted against pH.

The following procedure was used to determine the effect of the ionic strength of the phosphate buffer on the reaction rate of xenon trioxide with *trans*-cinnamaldehyde. Aliquots (200 ml.) of the stock solution ($8.0 \times 10^{-3} M$) were pipeted into three volumetric flasks (250 ml.). Monobasic potassium phosphate was added to each in sufficient quantity to make the final phosphate buffer concentration 0.75 *M*, 0.50 *M*, and 0.20 *M*, respectively. The solutions were then adjusted to pH 5.0 with potassium hydroxide and diluted with water to bring the volume to 250 ml. An aliquot (100 ml.) of each solution was then reacted with xenon trioxide solution (200 μ l., 0.09 *M*) at zero time at a temperature of $30 \pm 0.1^\circ$. The reaction rates were determined as described previously. The reaction was followed to at least 50% completion. The rate constants and half-lives were calculated. The rate constants were then plotted against the ionic strength of the solution on semilogarithmic graph paper.

RESULTS AND DISCUSSION

The results of the oxidation of *cis*- and *trans*-cinnamyl alcohol, *trans*-cinnamaldehyde, and *trans*-cinnamic acid by xenon trioxide are shown graphically in Fig. 1, where the number of millimoles of xenon trioxide consumed is plotted against the number of millimoles of the alcohol, aldehyde, and acid taken. In each case the relationship is linear. Upon extrapolation, the straight line plots intercept the ordinate above the origin. The slope and y -intercept for each plot were calculated by least-square regression analysis. The analytical data are recorded in Table I. In the stoichiometric studies the substrates were oxidized completely to carbon dioxide and water. Therefore, where the x - and y -axis values represent the millimoles of substrate and xenon trioxide consumed, respectively, the slope for each plot corresponds to the stoichiometric ratio of the reactants involved in the oxidation. The ratio is characteristic of the compound oxidized. The theoretical values for the stoichiometric ratios (Table I) were derived on the basis of the chemical equation representing the complete oxidation of the particular compound. This is illustrated with the following example where 7 moles of xenon trioxide are required to oxidize 1 mole of cinnamaldehyde:



The experimental results show less than 4% deviation from the theoretical values.

Intercepts with the ordinate greater than zero were noted in all cases. This deviation may be attributed to secondary processes resulting from the formation

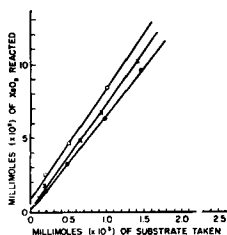


Fig. 1—The stoichiometry of cinnamyl alcohol, *trans*-cinnamaldehyde, and *trans*-cinnamic acid with xenon trioxide. Key: ○, cinnamyl alcohol; X, *trans*-cinnamaldehyde; ●, *trans*-cinnamic acid.

TABLE I—SUMMARY OF THE STOICHIOMETRIC DATA

Substrate	y -Intercept, moles $\times 10^3$	Slope, m	Theoretical Slope, m' ^a	Deviation, %
Cinnamyl alcohol	0.9169	7.423	7.333	1.23
<i>trans</i> -Cinnamaldehyde	0.1971	7.204	7.000	2.91
<i>trans</i> -Cinnamic acid	0.2314	6.415	6.667	3.77

^a Based on compound being completely oxidized to carbon dioxide and water.

of lower oxidation state xenon species such as xenon monoxide, XeO, which can react with water to form peroxide, and this in turn is capable of reacting with xenon trioxide to form xenon dioxide, XeO₂. Xenon dioxide is highly unstable, decomposing to its component elements spontaneously. Appelman (3) has reported on the reactive properties of XeO and XeO₂ in connection with their interaction with XeF₂. It is not likely that upon binary collision the substrate will give up six electrons in one step to a single xenon trioxide molecule. A stepwise transfer of electrons probably occurs which will result in lower oxidation states of xenon capable of reacting with water as indicated above and thus producing intercepts greater than zero.

The kinetic study of the oxidation of *trans*-cinnamaldehyde with xenon trioxide was carried out under such conditions that the quantity of aldehyde taken was in large excess relative to xenon trioxide. Therefore it can be assumed that the concentration of aldehyde remained essentially constant throughout the course of the reaction. The reaction rate may be expressed by the equation:

$$-\frac{d(\text{XeO}_3)}{dt} = k(\text{XeO}_3)$$

A plot of the xenon trioxide concentration remaining in the reaction mixture *versus* time on semilogarithmic graph paper should be a straight line. This is shown in Fig. 2. Thus, the reaction is first order with respect to xenon trioxide. In view of the fact that the determinations were conducted under conditions where the concentrations of the species other than xenon trioxide were kept essentially constant, the reaction is actually pseudo-first order.

In order to establish the reaction order with respect to *trans*-cinnamaldehyde, a series of experiments was conducted in which the concentration of the aldehyde was varied while the initial concentration of xenon trioxide was kept constant. The data, as shown in Table II, indicate no significant change in the reaction rate as the concentration of the aldehyde was increased three fold. This indicates that

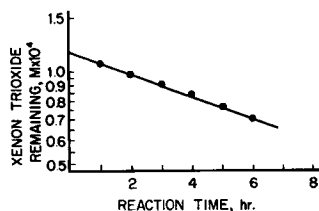


Fig. 2—Rate plot for the oxidation of *trans*-cinnamaldehyde with xenon trioxide at pH 5.35.

TABLE II—THE FIRST-ORDER REACTION RATES FOR THE OXIDATION OF *trans*-CINNAMALDEHYDE WITH XENON TRIOXIDE ($1.0802 \times 10^{-4} M$) AT $30 \pm 0.1^\circ$ AND pH 5.35

Initial Concentration of <i>trans</i> -Cinnamaldehyde, moles $\times 10^4$	Rate Constant, $\times 10^2 \text{ hr.}^{-1}$	Half-life, hr.
21.4495	8.74	7.92
32.1743	8.22	8.42
42.8990	8.14	8.51
53.6238	8.53	8.12
64.3485	8.20	8.45

the reaction is independent of the aldehyde concentration within the concentration range studied. The reaction is, therefore, zero order with respect to the aldehyde. A possible explanation may be that the concentration of the aldehyde already present in the reaction mixture was so great that any further increase in concentration did not elicit a discernible effect on the reaction rate. Another possibility may be the heterogeneity of the mixture. For instance, the solubility of *trans*-cinnamaldehyde is about $1.08 \times 10^{-2} M$; the concentration used in this study ranged from 2 to $6 \times 10^{-3} M$. However, because of the monopotassium phosphate, some of the aldehyde may be "salted out" of solution. Although this would result in a lower concentration of aldehyde in solution, the "salted out" aldehyde would serve as a source of replenishment for that consumed by the oxidant and thus maintain its concentration in solution nearly constant.

The reaction rates for the oxidation of *trans*-cinnamaldehyde with xenon trioxide were determined in $0.2 M$ phosphate buffer at various pH values ranging from 1 to 7. The study was restricted to acid conditions since xenon trioxide tends to disproportionate in alkaline media to form perxenate and xenon gas. A plot of the rate constant variation with pH is shown in Fig. 3. It was noted that the reaction rate increased with pH to a maximum at pH 4.3, then decreased to a minimum at pH 5.5, and increased sharply above pH 5.5. The pH profile is in general agreement with the observations of Jaselskis and Vas (4) who studied the oxidation of certain diols with xenon trioxide. They noted poorer oxida-

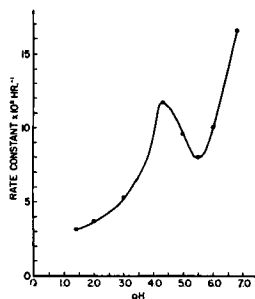


Fig. 3—Effect of pH on rate of oxidation of *trans*-cinnamaldehyde with xenon trioxide.

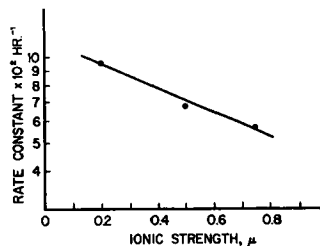


Fig. 4—Effect of buffer concentration on the rate of reaction of *trans*-cinnamaldehyde with xenon trioxide at pH 5.35.

tion at lower pH values. The maximum at pH 4.3 as shown in Fig. 3 cannot be fully explained at this time. The reaction at this pH may be catalyzed by the H_2PO_4^- species of the phosphate buffer system. Calculations of the ionic species present as a function of pH indicate that the concentration of this ion is maximal at pH 4.6. The species of the xenon trioxide in aqueous solutions may also vary with pH and may represent an important factor in the oxidation reactions studied here. Further studies are required to clarify more fully the pH effects noted here.

The effect of buffer concentration on the rate of oxidation of *trans*-cinnamaldehyde is shown graphically in Fig. 4. The reaction rate was found to decrease with increase in buffer concentration. In calculating the ionic strength for the reaction mixtures, the contribution of species other than the buffer was negligible. The effect of buffer concentration on the reaction rate may be attributed to a primary salt effect. Since the activity coefficients of the ions present may be affected by increased ionic strength, this may be reflected in a change in rate constant.

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Keyphrases

Xenon trioxide oxidation—organic compounds
Cinnamic compounds—xenon trioxide oxidation
Stoichiometry—cinnamic compounds, oxidation
Kinetics—cinnamic compounds, oxidation
pH dependency—oxidation rate
Ionic strength, phosphate buffer—oxidation rate