$$P_1 = \frac{P}{X_0}\left[X_0 e^{-\pi T} + \frac{k_0}{\pi}(1 - e^{-\pi T})\right] \quad \text{(Eq. 24)}$$

$$A_1 = \frac{A}{X_0}\left[X_0 e^{-\alpha T} + \frac{k_0}{\alpha}(1 - e^{-\alpha T})\right] \quad \text{(Eq. 25)}$$

$$B_1 = \frac{B}{X_0}\left[X_0 e^{-\beta T} + \frac{k_0}{\beta}(1 - e^{-\beta T})\right] \quad \text{(Eq. 26)}$$

After estimation of P, A, B, π, α, and β, the other pharmacokinetic parameters can be computed using the conventional methods (2) for blood concentration data obtained after a single rapid intravenous injection of the drug.

Occasionally, it is desirable to inject a drug slowly at a constant rate until the desired steady-state blood drug concentration is achieved. However, the time required (about $7 \times t_{1/2\beta}$) to obtain steady-state blood concentrations will be quite long for a drug with a long half-life. It may then be convenient to administer an intravenous priming dose simultaneously with the continuous infusion to obtain steady-state conditions rapidly (3, 4). The relationships derived in this paper are useful for the estimation of pharmacokinetic parameters in these situations.

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Hydrolysis Mechanism of 7-Acetylacroninium Perchlorate, a Novel Prodrug of Acronine

A. J. REPTA ˣ, J. R. DIMMOCK *, BO KREILGÅRD ‡, and JAMES J. KAMINSKI §

Abstract □ 7-Acetylacroninium perchlorate was hydrolyzed at 25° by both water enriched with ^{18}O-labeled water and by unenriched water. The acronine obtained was examined by mass spectrometry, which indicated the unusual fact that hydrolysis of this ester proceeded by aryl oxygen cleavage to the extent of about 30% under those mild conditions.

Keyphrases □ 7-Acetylacroninium perchlorate—hydrolysis mechanism □ Acronine prodrug—7-acetylacroninium perchlorate, hydrolysis mechanism □ Prodrugs—7-acetylacroninium perchlorate, hydrolysis mechanism □ Hydrolysis—7-acetylacroninium perchlorate, mechanism determined

The alkaloid acronine[1] (I) has activity against a wide range of tumors (1), but a major problem associated with its administration has been its low water solubility, only about 2–3 mg/liter (2).

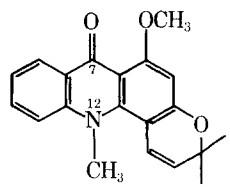
BACKGROUND

Attempts have been made to increase acronine solubility in water by coprecipitating the drug with povidone (3), using a mixture of an organic solvent and water (4), and complexation with various ligands (4). All of these methods failed to increase the water solubility of acronine to a concentration of 30 mg/100 ml, which was the value desired for intravenous administration.

Another approach was based on the fact that, in the presence of acid, protonation of the 7-oxygen of acronine resulted (II) (4). Thus, in preparing an acronine prodrug, acylation of the 7-hydroxy group of an acroninium salt would be expected to produce a 7-acyloxyacroninium salt with increased water solubility compared to acronine and yet be capable of regenerating acronine *in vivo*. 7-Acetylacroninium perchlorate (III) was prepared and is over 100 times more water soluble than acronine; *in vitro*, it is converted quantitatively to acronine. However, the prodrug suffers from the disadvantage of being hydrolyzed too rapidly [$t_{1/2} \sim 20$ min under ambient temperatures (18–25°) in nonirritating, nontoxic, and water-miscible media] to be of clinical use (5).

The rapid solvolysis of III may be due, in part, to aryl oxygen fission, since modification of the alkyl moiety of the ester group of analogs of III reportedly yielded compounds with nearly identical hydrolysis rates (4). In addition, the reaction of III with both aniline and mercaptide ions resulted in the formation of the corresponding 7-anil and 7-thioketones.

The purpose of this work was to determine whether hydrolysis by aryl oxygen fission would occur to an appreciable extent under mild conditions. Such information is of interest from a general standpoint and might also prove useful in the design of related prodrugs with improved stability properties.



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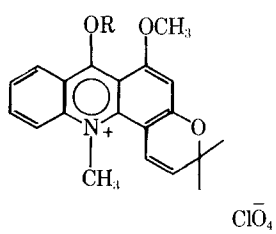
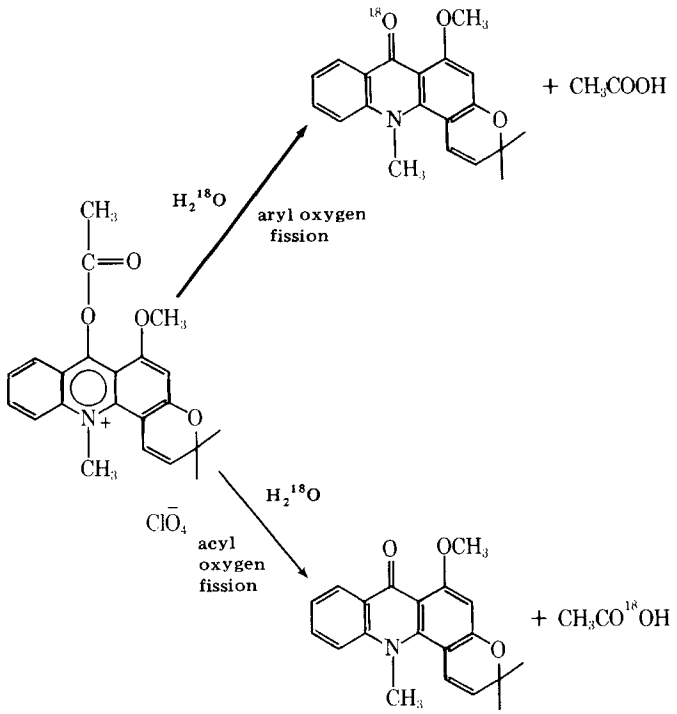


II: R = H
III: R = $COCH_3$

[1] Referred to as acronycine previously.



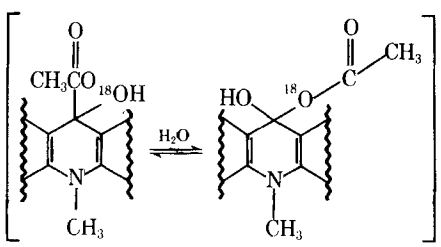
Scheme I

One method of determining the extent to which aryl oxygen cleavage occurs is hydrolysis of the ester in the presence of ^{18}O-labeled water. If acyl oxygen fission solely occurs, the product formed (acronine) will be devoid of any oxygen atoms containing oxygen-18. Alternatively, if aryl oxygen cleavage of the ester occurs, the acronine formed will contain oxygen-18 in the 7-position (Scheme I).

RESULTS AND DISCUSSION

7-Acetylacroninium perchlorate (III) was hydrolyzed in water enriched with 38.0 atom % $H_2{}^{18}O$ and also in unriched water to give products designated A and B, respectively. These products were examined by mass spectrometry. Acronine has a parent ion of *m/e* 321, and acronine containing oxygen-18 in the 7-position has a parent ion of *m/e* 323. Spectra from *m/e* 240 to 324 of both A and B revealed that the intensity of the ion at *m/e* 323 (expressed as a percentage of the total of the ion intensities from *m/e* 240 to 324) was approximately three times greater with A than with B. Since an additional experiment determined that no appreciable exchange between the 7-oxygen of acronine and ^{18}O-labeled water occurred, aryl oxygen cleavage of the ester was indicated.

The question arises as to the extent of aryl ester cleavage during the hydrolysis of III. One solution to this problem is to compare the intensities of pairs of ions representing the same molecular fragment, one of which contains oxygen-16 and the other oxygen-18 in the 7-group. Thus, for A and B, the intensities of the ions at *m/e* 323 and 321 of both spectra were measured; the relative intensity of the *m/e* 323 ion was expressed as a percentage according to the expression $100(M + 2)/[M + (M + 2)]$, where M + 2 and M are the intensities of the ions at *m/e* 323 and 321, respectively. The difference between the figures obtained from the spectra of A and B gives the excess atom percentage of oxygen-18. From this figure, the percentage of aryl oxygen cleavage can be calculated (see *Experimental*).



Scheme II

The ions at *m/e* 308 and 306 were also compared, these fragments arising by loss of 15 mass units from the parent ion. Comparison of the ratio of the intensities of the ions *m/e* 323/321 and 308/306 indicated that acyl oxygen cleavage occurred to the extent of nearly 30%.

The fact that only 30% of the III hydrolysis occurred *via* the aryl oxygen route was somewhat surprising in view of previous findings (4) that the hydrolytic rates of more sterically hindered species, such as pivalylacroninium ions, were very similar to those of the acetyl derivative. This finding had suggested that aryl oxygen fission might be a major hydrolytic pathway. With III, a facile nucleophilic attack of aniline and mercaptide ions occurred (which, with aniline, was a quantitative reaction) to yield the corresponding 7-derivative. The results may be due to the greater nucleophilicity of these compounds relative to water.

With several other 7-acyl analogs of III, the hydrolysis rates also were similar to those of III (4). This observation is difficult to rationalize in light of the present results, which imply that acyl oxygen cleavage is the major route for hydrolysis in such a series of esters and that changes in the steric bulk of the acyl component of the ester would be expected to affect hydrolysis rates significantly. One possible contributing factor in this dilemma might be the existence of a tetrahedral intermediate having a sufficient lifetime to permit the exchange of the acyl group between the labeled and unlabeled oxygen atom (Scheme II).

The existence of such an intermediate could reduce the label at the 7-position by a factor of two at most, and this maximum would be achieved only if total equilibration of the sort shown in Scheme II occurred. In that case, half of the ^{18}O-label would be lost as ^{18}O-acetate. Consequently, it is possible that the reaction pathway involving aryl oxygen fission might have occurred to a maximum extent of about 60%, although it seems unlikely that the intermediate would be sufficiently stable to result in statistically complete mixing of the labeled atom. Therefore, an intermediate value between 30 and 60% would be more reasonable.

Nevertheless, the fact that the aryl oxygen fission of the ester linkage occurs to the extent of 30% or more under such mild conditions is unusual and attests to the abnormal properties of the ester linkage of the 7-acylacroninium prodrugs. These results also suggest that utilization of a prodrug approach based on the modification of the ester group is unlikely to increase greatly the prodrug stability since the degradation pathways appear to involve components of both acyl and aryl oxygen fission.

EXPERIMENTAL[2]

A freshly synthesized sample of 7-acetylacroninium perchlorate (III) (0.00556 g) (4, 5), shown by spectrometry (6) to contain 72% of the ester III[3], was added to a tube containing 38.0 atom % $H_2{}^{18}O$ (5.0 ml) and placed in a sonic bath[4] for 18 min. The tube was attached to the spindle of a constant-temperature bath at 25° and agitated by rotating slowly for 19 hr. The contents of the tube were then centrifuged[5] for 25 min, and the precipitate (A) was collected, dried, and weighed (0.00328 g).

The experiment was repeated using III (0.00524 g) and water (5.0 ml) to give a product (B) weighing 0.00355 g. The mass spectra from *m/e* 240 to 324 of products A, B, and acronine were determined using an expanded scale recorder. The intensities of the ions were measured, and peaks shorter than 5 mm were ignored. The abundance of ions was corrected for background intensities.

In the first experiment, the mass spectra of A, B, and acronine were determined at 120° under identical conditions, and the intensity of the *m/e* 323 ion was expressed as a percentage of the sum of the intensities of the ions from *m/e* 240 to 324. Five determinations for A and acronine and four for B were made. The relative intensity of the ion at *m/e* 323 for A, B, and acronine was 1.418% (range of 1.391–1.441%), 0.519% (range of 0.501–0.545%), and 0.513% (range of 0.483–0.552%), respectively.

In another experiment, acronine was placed in 5.0 ml of water containing 38.0 atom % $H_2{}^{18}O$ and rotated for 19 hr at 25° as described previously. The intensity of the ion at *m/e* 323 compared to the intensities of the ions from *m/e* 240 to 324, expressed as a percentage for both the compound suspended in ^{18}O-labeled water for 19 hr and an authentic sample of acronine, was 0.614% (range of 0.583–0.654%) and 0.575% (range

[2] Mass spectra were determined by Mr. R. Drake of the Department of Chemistry, University of Kansas, by direct sample introduction using a Varian CH-5 instrument operating at 70 ev. Water enriched with ^{18}O-labeled water contained 40.0 atom % ^{18}O obtained from Bio-Rad Laboratories, Richmond, CA 94604.

[3] The remaining 28% of the sample consisted of acronine and acroninium perchlorate.

[4] Bransonic model 220.

[5] International clinical model CL.

of 0.523–0.603%), respectively. Five mass spectra for each compound were determined at 120°.

Ten spectra from *m/e* 240 to 324 were then run at 111° for A and B under identical conditions. The intensities of the ions at *m/e* 323 were compared to those at *m/e* 321 for A and B using the formula $[(M + 2)/M + (M + 2)] \times 100\%$, where M + 2 and M were the intensities of the ions at *m/e* 323 and 321, respectively. The values for A and B were 11.3 and 3.4%, respectively. The percentage hydrolysis proceeding *via* aryl oxygen cleavage, 28.9%, was obtained from the expression $[(11.3 - 3.4)/(38 \times 0.72)] \times 100$, 38 and 0.72 being the atom percent of $H_2{}^{18}O$ and the fractional purity of II, respectively. The intensities of the ions at *m/e* 308 and 306 were evaluated similarly, and the percentage of aryl ester cleavage was 28.0%.

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New Rapid Determination of Pyridoxal Phosphate Using Tyrosine Phenol-lyase

G. G. MEADOWS, L. BOZE, and G. W. ELMER ˣ

Abstract □ A rapid, specific, and precise spectrophotometric assay for the determination of pyridoxal phosphate is described.The assay allows for the determination of the cofactor between 0.1 and 1.0 μg/ml. Its applicability to pyridoxal phosphate in biological fluids was demonstrated by a determination of the plasma half-life in BDF_1 mice. Pyridoxal phosphate is absorbed rapidly from the peritoneal cavity and cleared from the plasma with a half-life of about 15 min.

Keyphrases □ Pyridoxal phosphate—spectrophotometric analysis, biological fluids □ Spectrophotometry—analysis, pyridoxal phosphate in biological fluids □ Enzyme cofactors—pyridoxal phosphate, spectrophotometric analysis in biological fluids

Pyridoxal phosphate is a cofactor for several enzymes that participate in amino acid metabolism (1). Because of this metabolic significance, there is considerable interest in quantitating both suboptimal and elevated plasma pyridoxal phosphate levels. The latter interest stems from the observation that megadoses of pyridoxine have beneficial therapeutic effects in certain vitamin-responsive genetic diseases (2, 3). Increased circulating levels of pyridoxal phosphate may also be of benefit in enhancing the antitumor activity of several pyridoxal phosphate-dependent enzymes such as methioninase (4, 5) and tyrosine phenol-lyase (6).

To elevate pyridoxal phosphate levels in mice in a previous study (6), large amounts of the cofactor were injected. To quantitate and follow the fate of exogenously administered pyridoxal phosphate in mice, a rapid assay utilizing tyrosine phenol-lyase was developed. Although other enzymatic assays specific for pyridoxal phosphate were reported (7–9), they are hampered by the fact that radionuclides and/or extraction of the cofactor are required. These procedures can be both time consuming and costly. The described assay utilizes pyridoxal phosphate-dependent tyrosine phenol-lyase in a coupled reaction with lactate dehydrogenase and NADH. Pyridoxal phosphate levels can be easily and rapidly ascertained by spectrophotometric measurements.

EXPERIMENTAL

Materials—Tyrosine phenol-lyase was purified from *Erwinia herbicola* (ATCC 21434) as described (6). When purified by this method, the enzyme has less than 2% of maximal activity in the absence of pyridoxal phosphate. Pig heart lactate dehydrogenase[1], A grade pyridoxal 5′-phosphate[1], and NADH[1] were used in the coupled assay. All other reagents were analytical grade.

Pyridoxal Phosphate Assay Procedure—Tyrosine phenol-lyase catalyzes tyrosine degradation to pyruvate, phenol, and ammonia. The enzyme activity was determined using the continuous spectrophotometric assay previously reported (6), except that the enzyme was preincubated with the pyridoxal phosphate, lactate dehydrogenase, and NADH for 10 min at 37° prior to addition of tyrosine. The assay measures the formation rate of pyruvate by a coupled reaction using lactate dehydrogenase and NADH. Oxidation of NADH is followed spectrophotometrically. One international unit (IU) of enzyme activity is defined as the amount of enzyme that catalyzes the formation of 1 μmole of product/min.

An adaptation of this assay was used for pyridoxal phosphate. Samples containing pyridoxal phosphate (0.5 ml) were preincubated for 10 min with 0.05 IU of tyrosine phenol-lyase (0.5 ml), 200 μmoles of NADH (0.5 ml), and 1.0 IU of lactate dehydrogenase (0.5 ml). Then 2.5 μmoles of

[1] Calbiochem, Los Angeles, Calif.