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Enol esters as potential prodrugs III. * Stability and enzyme-mediated hydrolysis of enol esters of 6′-acetylpapaverin

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Summary

Based on results obtained previously from studies of some model α-acyloxystyrenes, 3 enol esters of 6′-acetylpapaverin (6′-AP) were selected for evaluation as potential prodrug forms of the quaternary antitumor agent, coralyne. Their in vitro stability in buffers, plasma and some tissue homogenates was studied. While exhibiting adequate stability in simple aqueous solutions, in biological fluids these enol esters reverted to coralyne apparently as a results of enzymatic catalysis. The results of this study indicate that enol esters of 6′-acetylpapaverin exhibit properties which suggest pharmaceutical utility as prodrugs for enhancing the intracellular levels of coralyne in brain and other tissues.

Introduction

The rationale behind the use of enol esters as potential prodrugs of enolizable ketones has been discussed in a previous communication (Patel and Repta, 1980). It was shown that α-acetyloxystyrene possessed sufficient aqueous stability and was a good substrate for the esterases of human and rat plasma and tissue homogenates. In subsequent work (Patel and Repta, 1981) the results of the relative rates of hydrolysis of several model α-acyloxystyrenes in aqueous media at various pH and temperatures and in biologically derived media were presented. It was demonstrated

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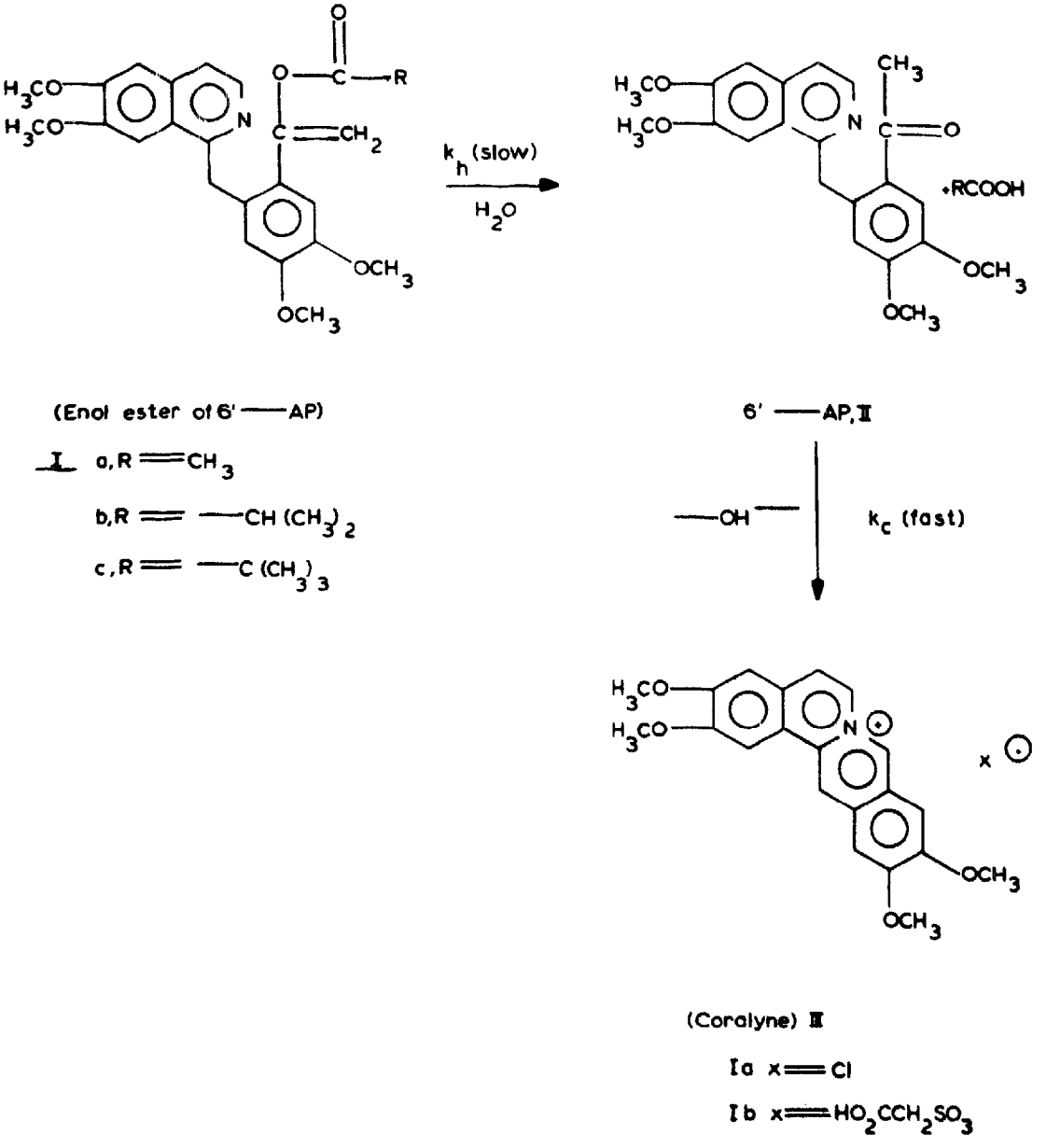
that the rates of hydrolysis in the biological and simple aqueous media could be altered appreciably by a judicious choice of the acyl functions. While the model enol esters reverted relatively rapidly and completely to the parent ketone in the biological media, all were quite stable in aqueous buffers.

This paper reports the in vitro rates of hydrolysis in aqueous and biological media of 3 enol esters of 6′-acetylpapaverin which itself is a very unstable prodrug of the experimental antineoplastic agent, coralyne (Cho et al., 1975).

Rationale for the choice of the 6′-AP enol esters (I)

Previous work (Cho et al., 1975) had shown that 6′-acetylpapaverin (6′-AP) II was an uncharged species which rapidly and spontaneously cyclized to yield the



quaternary species, coralyne (III), an antineoplastic agent. It has also been reported that coralyne is not distributed to brain after i.v. dosing (Plowman et al., 1976). Presumably, this is due to the quaternary nature of the agent.

Our premise is that pharmaceutically stable prodrug forms of 6′-AP which revert in vivo might result in delivery of 6′-AP to brain tissue where it could cyclize to the quarternary species which might be retained in that tissue due to poor efflux across the blood–brain barrier. Enol esters of 6′-AP appeared to be a reasonable prodrug type.

The results of the hydrolytic studies of some model α-acyloxystyrenes in buffered aqueous media and in tissue homogenates and fluids (Patel and Repta, 1980b) indicated that enol esters may be potentially useful bioreversible derivatives of ketones. Furthermore, that study demonstrated that among the alkyl model compounds, the aqueous and enzyme-mediated rates of hydrolysis of the acetate, isobutyrate and pivalate enol esters differed significantly.

The conversion of an enol ester of 6′-AP involves two steps as shown in Scheme I, with the hydrolysis of the ester (k_h) being the rate-determining step in the sequence.

The relative rates of in vivo hydrolysis of enol esters might be expected to show rank order correlation with in vitro rates in biological media. Furthermore, it can be reasonably speculated that the in vitro rate (and sites) of hydrolysis might significantly affect the efficiency with which coralyne would be delivered to brain tissue. Therefore, the acetate, isobutyrate and pivalate enol esters of 6′-AP were selected for in vitro evaluation of their pharmaceutical stability as well as for their susceptibility to enzyme-catalyzed hydrolysis.

Materials and methods

Materials

Unless otherwise stated all chemicals were analytical grade reagents. Coralyne chloride (IIIa) (NSC no. 96349) and coralyne sulfoacetate (IIIb) (NSC no. 154890) were provided by the National Cancer Institute, Bethesda, MD and were used without further purification.

Silica gel CC-7 (Mallinckrodt Chemical Works, St. Louis, MO) was used for all purification and separations of enol esters by column chromatography. All thin-layer chomatography (TLC) was done on plastic silica gel TLC sheets (Polygram SILG/UV 254, Brinkman Instruments, Westburg, NY) with fluorescent indicator.

Human serum albumin, fraction V, was obtained in purified form from United States Biochemicals, Cleveland, OH. Male Sprague–Dawley rats weighing 225–250 g were obtained from Harlan Sprague–Dawlev, Madison, WI. Recovered human plasma was obtained from the Community Blood Center, Kansas City, MO.

All glassware used in synthesis of the enol esters was dried at 120°C for at least 12 h, assembled hot and cooled under dry nitrogen. Transfers of liquids were made using dry glass syringes. Acid anhydrides were freshly distilled using an all-glass still. Dicyclohexylamine was distilled under reduced pressure prior to use. Tetrahydrofuran (THF) was distilled from lithium aluminium anhydride and used immediately.

Methods

Synthesis

6'-Acetylpapaverin (II). 6'-Acetylpapaverin (6'-AP) was prepared from coralyne sulfoacetate (CSA) or coralyne chloride by the procedure given by Schneider and Schroeter (1920).

6'-AP enol esters. All esters were prepared by the same general procedure which involved the reaction of 6'-AP with butyl lithium to form the lithium enolate of 6'-AP which was then reacted with the desired acid anhydride to obtain the apropriate enol ester of 6'-AP. Specific details are illustrated in the following example.

Synthesis of 6'-AP enol acetate (Ia). To a solution of dicyclohexylamine (0.7 ml, 3.6 mmol) in dry THF (100 ml), under an atmosphere of dry nitrogen and cooled in a bath at −70°C (dry ice/acetone), an aliquot (2 ml, 3.2 mmol) of a solution of *n*-butyl lithium (1.6 M) in hexane was added. The mixture was stirred at −70°C for 10 min and 6'-AP (1 g, 2.6 mmol) dissolved in THF (~20 ml) was added slowly to the reaction mixture. Subsequently, dry THF (~60 ml) was added to the reaction mixture, which turned cherry-red due to the formation of lithium enolate of 6'-AP. The solution was stirred for 15 min, and acetic anhydride (1.25 ml, 13 mmol) dissolved in dry THF (20 ml) was then added dropwise. After the addition was complete, the mixture was stirred under nitrogen and allowed to warm slowly to room temperature after which the reaction mixture was evaporated to dryness under reduced pressure. The residue was mixed with diethylether (500 ml) and transferred to a one-liter separatory funnel. The ether solution was washed with cold (~5°C) water (200 ml), followed by a cold saturated solution sodium bicarbonate (2 × 200 ml) and then again with cold water (200 ml). The ether layer was separated, dried over anhydrous sodium sulfate (10 g) for 15 min, filtered and the sodium sulfate washed with more ether. The combined filtrate and wash were evaporated to dryness under reduced pressure. The residue was dissolved in the minimum required volume of chloroform, applied to a column of silica gel (100 g) and eluted with chloroform. Most of the coralyne produced as a result of cyclization of 6'-AP and other side-products of the reaction remained on the column. 6'-AP enol acetate was collected in the eluent. The fractions containing the product were combined, evaporated to dryness under reduced pressure and the residue dissolved in the minimum volume of diethyl ether. Solid 6'-AP enol acetate was obtained after in vacuo removal of the ether; yield was ~40%, m.p. 52–56°C (decomp.).

The product moved as a single spot on TLC (R_f 0.47 on silica) using acetone. Elemental analysis was consistent with the composition $C_{24}H_{25}NO_6$ and the 60 MHz nmr spectrum (in deuterochloroform) was consistent with the structure of Ia. The characteristic nmr data were: 2.05 (s, 3H), 3.55 (s, 3H), 3.88 (s, 3H), 3.9 (s, 3H), 4.0 (s, 3H), 4.7 (s, 2H), 5.2 (q, 2H) 6.4–8.4 (m, 6H).

When the above synthetic procedure was carried out using isobutyric anhydride, the solid was obtained in 20% yield and m.p. ~145–150 (decomp.). A single spot (R_f 0.52) was observed by TLC. Elemental analysis was in agreement with $C_{26}H_{29}NO_6$ [1] (Ib) as were the nmr data: 1.20 (d, 6H), 2.60 (m, 1H), 3.53 (s, 3H), 3.84

(s, 3H), 3.89 (s, 3H), 3.98 (s, 3H), 4.75 (s, 2H), 5.2 (q, 2H), 6.4–8.4 (m, 6H).

Similarly when pivalic anhydride was used, a solid (m.p. 168–172 (decomp.)) was obtained which showed only a single spot (R_f 0.54) by TLC. This material was characterized as Ic ($C_{27}H_{31}NO_6$) by elemental analysis [1] and by nmr: 1.25 (s, 9H), 3.5 (s, 3H), 3.8 (s, 3H), 3.87 (s, 3H), 4.0 (s, 3H), 4.75 (s, 1H), 5.15 (q, 2H), and 6.4–8.4 (m, 6H).

Kinetic studies

Stock solutions of 6′-AP enol esters were prepared in dry acetonitrile and the required volume of the enol ester of interest was mixed with aqueous buffers, plasma or tissue supernatants.

The initial enol ester concentration in all kinetic studies ranged from 1.8×10^{-5} to 3.2×10^{-5} M. The final concentration of acetonitrile in all the studies was always $\leq 0.5\%$ (v/v). No significant difference in the observed rate constants were seen when the volume of acetonitrile in the hydrolysis medium was varied from 1 to 3% (v/v). The rates of loss of 6′-AP and its enol esters in buffers and biological media were based on spectrophotometric monitoring of increasing absorbance at $\lambda = 299$ nm which corresponded to the formation of coralyne. A constant ionic strength was maintained at 0.3 M with sodium chloride for all buffers used.

Preparation of tissue homogenates

Sorenson's isotonic phosphate buffer, pH 7.4, was used to prepare tissue homogenates. The details of the method have been described (Patel and Repta, 1981).

Results and Discussion

Kinetic studies in buffered aqueous media

The rates of hydrolysis of 6′-AP enol esters followed pseudo-first-order kinetics in aqueous buffers at the various pH values and temperatures studied. These observations were similar to those of the model α-acyloxystyrenes (Patel and repta, 1980, 1981). The influence of pH and different buffers (at constant ionic strength) on the apparent hydrolytic rate constant for 6′-AP enol acetate at 60°C is shown in Table 1. The data in this table demonstrate the catalysis of the hydrolytic reaction by acetate, phosphate and borate buffers. However, the catalytic effects are not large and it appears that in each buffer the more catalytic species are those which could behave as general bases. These findings are similar to those reported previously for the hydrolysis of α-acyloxystyrenes (Patel and Repta, 1981).

The pH–rate profile for Ia is shown in Fig. 1. The data points are the buffer-independent (i.e. buffer concentration = 0) values shown in Table 1. At pH ≤ 2 and pH ≥ 8.5, specific acid and specific base catalysis, respectively, predominate as

[1] In both Ib and Ic, elemental analysis values (for carbon only) were slightly low. Increasing the oxidation time from 50 to 90 s increased the carbon values from 67.99 to 68.59 (theory 69.16) for Ib and from 67.47 to 68.48 (theory 69.66) for Ic. The H and N values obtained were unaffected by combustion time.

TABLE I

EFFECTS OF BUFFER CONCENTRATION AND pH ON THE APPARENT FIRST-ORDER RATE CONSTANT FOR THE HYDROLYSIS OF Ia IN AQUEOUS SOLUTIONS (μ=0.3, T=60°C)

Bufferspecies	Concentration	pH	$k \times 10^4$ (min^{-1})	pH	$k \times 10^4$ (min^{-1})
Borate	0	9.35	662 [a]	8.35	99 [a]
	0.010		690		109
	0.025		719		124
	0.060		764		150
	0.10		883		197
Phosphate	0	7.40	29.5	6.25	26.6 [a]
	0.010		31.5		28.1
	0.025		32.6		35.6
	0.060		38.0		46.1
	0.10		44.1		56.7
Acetate	0	5.05	8.3 [a]	3.80	1.3 [a]
	0.010		8.7		1.4
	0.025		9.7		1.2
	0.060		11.6		1.4
	0.10		13.5		1.4

[a] Estimated by extrapolation.

indicated by the nearly unit slopes in Fig. 1. The sigmoidal portion of the profile (pH ~4–7) may be attributed to the ionization of the nitrogen atom of the isoquinoline system. Overall, this pH profile is quite similar in shape to that reported for α-(N,N-dimethylamino)acetyloxystyrene (Patel and Repta, 1981).

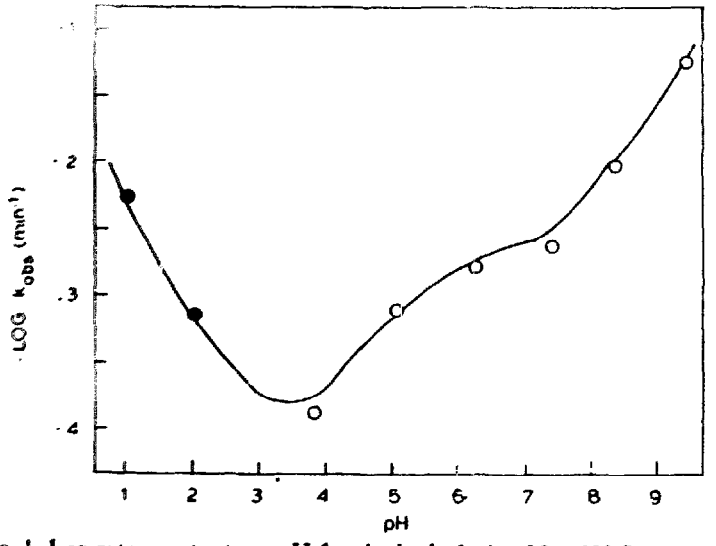


Fig. 1. Log rate constants vs pH for the hydrolysis of Ia (60°C, μ=0.3). The open symbols represent data points obtained from plots of k_{obs} vs buffer concentration when extrapolated to zero buffer concentration. The solid symbols (at pH 1.05 and 2.05) were obtained for aqueous hydrochloric acid solutions. The solid line was constructed using Eqn. 6 and the values given in Table 2.

The pH dependence of the observed rate constant, k_{obs} in Fig. 2 for 6′-AP enol acetate can be adequately accounted for by considering the 4 reactions in Eqns. 1–4 where 6′-APA and 6′-APA$^+$ represent the neutral and protonated forms of the ester, respectively.

$$6'\text{-APA}^+ + H^+ \xrightarrow{K_1} \text{Products} \quad (1)$$

$$6'\text{-APA}^+ \xrightarrow{k_2} \text{Products} \quad (2)$$

$$6'\text{-APA}^+ + OH \xrightarrow{k_3} \text{Products} \quad (3)$$

$$6'\text{-APA} + OH \xrightarrow{k_4} \text{Products} \quad (4)$$

with $[6'\text{-APA}]_T$ as the total concentration of 6′-AP enol acetate (i.e. $[6'\text{-APA}]_T = [6'\text{-APA}^+] + [6'\text{-APA}]$), the overall rate law for the hydrolysis of 6′-AP enol acetate may be written as

$$-d[6'\text{-APA}]_T/dt = k_1[6'\text{-APA}^+][H^+] + k_2[6'\text{-APA}^+] + k_3[6'\text{-APA}^+]$$
$$[OH^-] + k_4[6'\text{-APA}][OH^-] \quad (5)$$

where k_1 is the second-order rate constant for the specific acid catalyzed hydrolysis

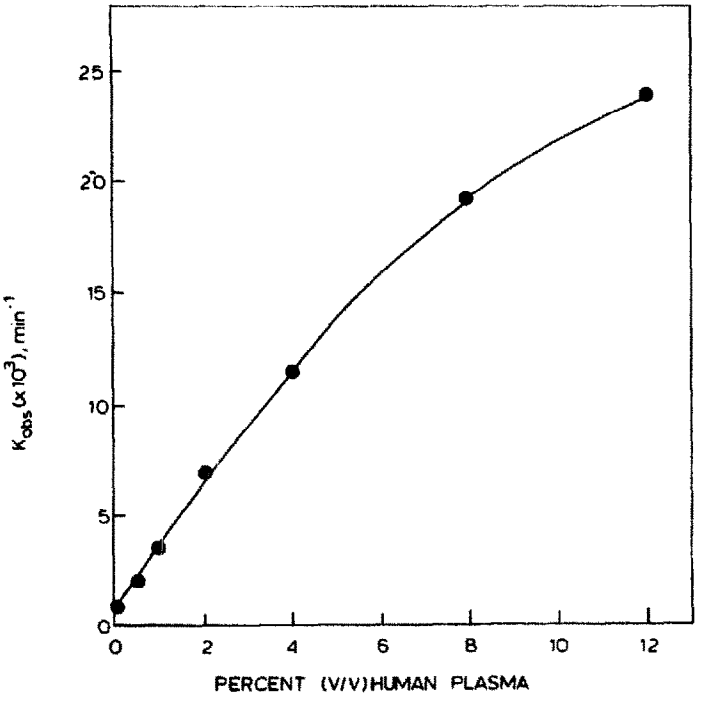


Fig. 2. Observed rate constant as a function of plasma concentration for the formation of coralyne upon hydrolysis of Ia at 37°C.

TABLE 2

THE ACID DISSOCIATION AND RATE CONSTANTS FOR THE HYDROLYSIS OF 6'-AP ENOL ACETATE (Ia) IN BUFFERED AQUEOUS SOLUTIONS AT 60°C, μ=0.3 M

Rate constant	Values [a]
k_1 (M min)$^{-1}$	61.6×10^{-3}
k_2 (M min)$^{-1}$	12.6×10^{-5}
k_3 (M min)$^{-1}$	66.1×10^{3}
k_4, min^{-1}	312.4
K_a, M	1.68×10^{-6}

[a] These values were obtained by computer-fitting of the data points in Fig. 1 according to Eqn. 6.

of 6'-APA$^+$, k_2 is the apparent first-order rate constant for the spontaneous hydrolysis of 6'-APA$^+$, and k_3 and k_4 are the second-order rate constants for the specific base catalyzed hydrolysis of 6'-APA$^+$ and 6'-APA, respectively.

Expressing the fraction of the protonated and neutral enol ester in terms of the acid dissociation constant (K_a) and the activity of the hydrogen ion (a_{H^+}), Eqn. 6 is obtained.

$$k_{obs} = (k_1[H^+] + k_2 + k_3[OH^-])\left(\frac{a_{H^+}}{a_{H^+} + K_a}\right) + k_4[OH^-]\left(\frac{K_a}{a_{H^+} + K_a}\right) \qquad (6)$$

Using Eqn. 6 and the values for the various rate constants and the acid dissociation constant shown in Table 2, the solid line in Fig. 1 was generated and found to provide a good fit for the data.

While the magnitudes of k_{obs} for the three 6'-AP enol esters would not be identical due to steric (and perhaps other) differences between the acetyl, isobutyryl and pivaloyl groups, it was expected that because of the structural similarities between Ia, Ib and Ic, the pH–rate profiles for Ib and Ic would be quite similar in shape to that shown in Fig. 1. Consequently, the pH–rate profiles of Ib and Ic were not determined.

Relative rates of hydrolysis of 6'-AP enol esters in alkaline media. The relative rates of hydrolysis of the acetate, isobutyrate and pivalate enol esters of 6'-AP were

TABLE 3

RATE CONSTANTS [a] AND HALF-LIVES FOR THE HYDROLYSIS OF 6'-AP ENOL ESTERS IN 0.1 M BORATE BUFFER, pH 9.35, μ=0.3 AT 60°C

6'-AP enol ester	$k\times10^3$ (min^{-1})	$t_{1/2}$ (min)
Ia (acetate)	65.4	11
Ib (isobutyrate)	27.6	25
Ic (pivalate)	5.78	120

[a] From rate of appearance of coralyne.

TABLE 4

EFFECT OF TEMPERATURE ON THE OBSERVED RATE CONSTANTS FOR HYDROLYSIS OF Ia IN AQUEOUS PHOSPHATE (0.025 M) BUFFER (pH 7.4, $\mu = 0.3$)

T (°C)	$k_{obs} \times 10^4/(min^{-1})$
40	5.62
50	13.7
60	34.3
70	70.1

studied in borate (0.1 M) buffer (pH 9.35, $\mu = 0.3$) at 60°C. The observed first-order rate constants and corresponding half-lives for the 3 enol esters are presented in Table 3. As expected these data indicate that increased steric crowding around the carbonyl carbon leads to a decrease in the rate of alkaline hydrolysis. Substituting the *t*-butyl group (Ic) for methyl (Ia) leads to ~ 10-fold increase in the observed rate constant. Similar observations were reported by Patel and Repta (1981) for the hydrolysis of the model α-acyloxysytrenes in alkaline media. Since, as stated above, the pH–rate profiles for Ia, Ib and Ic would be expected to be similar except for translation due to different values of the specific rate constants, it might be anticipated that the relative stability of all 3 of the 6′-AP enol esters over the entire pH-range would retain the rank order stability observed at pH 9.35, i.e. the stability of Ic > Ib > Ia.

Temperature effects. The rates of hydrolysis of Ia were determined at 40, 50, 60 and 70°C in phosphate (0.025 M) buffers at physiological pH (7.4). The rate constants obtained are given in Table 4. A plot of the obtained values of k_{obs} vs reciprocal temperatures (°K) was linear ($r^2 = 0.9998$) and an observed activation energy (E_a^{obs}) of 18.15 kcal/mol was calculated. However, the reaction rate at pH 7.4 involves primarily the reactions shown in Eqns. 3 and 4, both of which are hydroxide ion-catalyzed. When corrected for the heat of ionization of water (i.e. 13.05 k_{cal}/mol (Harned and Hamer, 1933)), the adjusted apparent activation energy, E_a^{adj} was 5.1 k_{cal}/mol [2]. From the temperature dependence studies on Ia, a value of $k_{obs} = 1.30 \times 10^{-4}$ min^{-1} ($t_{1/2} \sim 90$ h) can be expected at 25°C. It is to be expected that the more sterically hindered enol esters—6′-AP enol isobutyrate (Ib) and pivalate (Ic)—would be even more stable than Ia under these conditions. Thus, it appears that 6′-AP enol esters are fairly stable in the neutral to slightly acidic aqueous media (maximum stability at pH ~ 3.5). Consequently, while such stability is desired in the pharmaceutical dosage form, it is obvious that enzymatic acceleration of the hydrolytic rate must occur in vivo in order to obtain rapid reversion to 6′-AP.

[2] It is recognized that the hydrolysis rate at pH 7.4 is a complex function of two rate constants and the ionization constant of Ia, all of which are temperature-dependent. Consequently, the linearity of the Arrhenius plot is fortuitous and the calculation of activation parameters may not have fundamental significance. However, in view of the linearity of the Arrhenius plot it appears reasonable and safe to use these data to obtain an estimate of the values of k_{obs} at pH 7.4 at ambient temperatures.

In vitro studies in biological media

The in vitro rates of conversion of 6′-AP and its enol esters to coralyne were followed spectrophotometrically in human and rat plasma and in the supernatant fractions of rat liver and brain homogenates. In these media the rates of appearance of coralyne from either 6′-AP or the enol ester followed pseudo-first-order kinetics.

Hydrolysis in plasma. The results of previousstudies (Patel and Repta, 1980, 1981) had indicated that the rates of hydrolysis of α-acyloxystyrenes in undiluted human plasma were quite rapid. Therefore, diluted plasma was used in this study. The data in Table 5 when compared with the rate data in non-biological media (Tables 1, 3 and 4) clearly demonstrate that components of plasma (presumably non-specific esterases) catalyze the hydrolysis of the 6′-AP enol esters. It is also quite clear from the data in Table 5 that the rate of conversion of 6′-AP enol esters to coralyne is significantly slower than the rate of cyclization of 6′-AP to coralyne (Scheme I). Thus, the rate-determining step in Scheme I is the rate of hydrolysis of the particular enol ester.

Table 5 also shows that the rates of loss of 6′-AP enol esters were significantly faster in rat plasma than in human plasma at similar concentrations. These results are similar to those found for the hydrolysis of α-acyloxystyrenes (Patel and Repta, 1981). Aldridge (1953) and Mendoza et al. (1976) have reported similar findings from the hydrolysis in human and rat sera of esters of *p*-nitrophenol.

The data in Table 5 demonstrate that an increased branching around the carbonyl carbon leads to a decrease in the rate of hydrolysis of the 6′-AP enol esters. As proposed earlier in related studies with the α-acyloxystyrenes (Patel and Repta, 1981) this is presumably due to a poorer fit of the substrate on the active site of the enzyme(s). 6′-AP enol pivalate, although a relatively poor substrate for the esterases in human plasma, was hydrolyzed comparatively rapidly in rat plasma, indicating a significant difference in the overall esterase activities of the plasma of the two species. Again, similar results were observed (Patel and Repta, 1981) for α-pivaloyloxystyrene.

TABLE 5

RATE CONSTANTS AND HALF-LIVES FOR THE CYCLIZATION OF 6′-ACETYLPAPAVERIN (6′-AP) (II) AND FOR THE HYDROLYSIS[a] OF 6′-AP ENOL ESTERS (Ia, Ib, Ic) IN VARIOUS CONCENTRATIONS OF HUMAN AND RAT PLASMA IN pH 7.4, ISOTONIC SORENSON'S PHOSPHATE BUFFER AT 37°C

Compound	5% human plasma		4% rat plasma	
	$k \times 10^3$ (min^{-1})	$t_{1/2}$ (min)	$k \times 10^3$ (min^{-1})	$t_{1/2}$ (min)
II	495	1.4	462	1.5
Ia	27.7	25	126	5.5
Ib	6.18	112	26.7	26
Ic	–[b]	–[b]	1.85	375

[a] The rate constants are actually based on formation of coralyne.
[b] Less than 10% hydrolysis in 24 h.

Effect of concentration of plasma

The rates of conversion of 6′-AP enol acetate and 6′-AP to coralyne were determined in several concentrations of human plasma (Figs. 2 and 3). As is evident from Fig. 2, the relationship between per cent plasma and k_{obs} values for the hydrolysis of Ia clearly is non-linear. In addition, the rate onstant for the cyclization of 6′-AP to coralyne was found to decrease non-linearly with increasing plasma concentration (Fig. 3).

There may be several possible explanations for the non-linear dependence shown in Fig. 2. Since there are numerous esterases and proteins (primarily albumin and globulins) present in human plasma, it is likely that at higher plasma concentrations there may occur intermolecular and perhaps even intramolecular associations among those macromolecules. Several kinds of interactions could occur including second- or higher-order association of protein(s) containing esterase sites with itself or with dissimilar proteins. it is also conceivable that the tertiary or quarternary structure of such proteins could vary as a function of plasma concentrations. Any or all of the above interactions could lead to a decrease in the accessability of those sites with esterase activity. Thus, as the concentration of plasma increases, a smaller fraction of the actual enzymatic (esterase) sites might be available for the catalysis of the hydrolysis of 6′-AP enol acetate. Such an occurrence would account for the negative deviation of the plot of k_{obs} vs per cent human plasma (Fig. 2).

In an attempt to ascertain whether or not protein interaction might be responsible for the non-linearity in Fig. 2, the hydrolytic behavior of 6′-AP enol acetate in human plasma containing added HSA, fraction V, was compared to several other related media. As is evident from the data presented in Table 6, the rate of

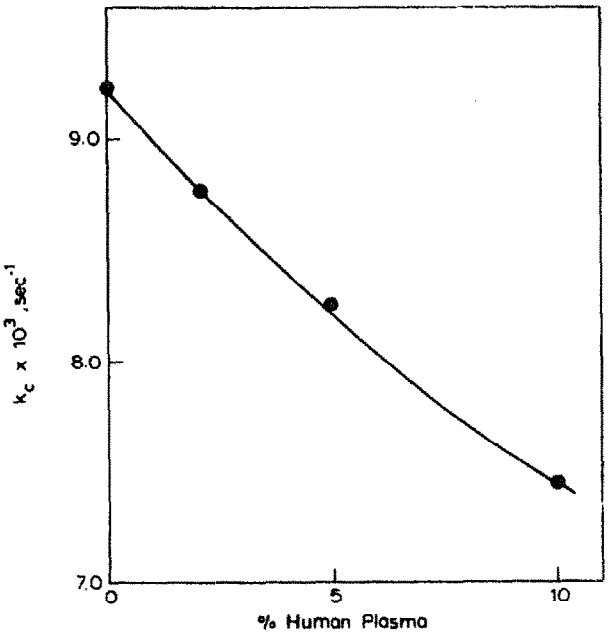


Fig. 3. Observed rate constant (k_c) for the cyclization of 6′-AP to coralyne ion in various concentrations of human plasma at 37°C. Isotonic Sorenson's phosphate buffer (pH 7.4) was used to dilute the plasma.

TABLE 6

RATE CONSTANTS AND HALF-LIVES FOR THE HYDROLYSIS[a] of 6'-AP ENOL ACETATE[a] AT 37°C IN 0.1 M PHOSPHATE BUFFER (pH 7.4, μ=0.3) WITH AND WITHOUT THE ADDITION OF 4% HUMAN PLASMA AND 0.42% HSA, FRACTION V

Hydrolysis medium	$k_{obs} \times 10^4$ (min^{-1})	$t_{1/2}$ (min)
0.1 M phosphate buffer	7.22	960
0.42% HSA, fraction V	20.4	340
4% human plasma	126	5[c]
4% human plasma + 0.42% HSA, fraction V	92.4	75

[a] The rates monitored were actually the rates of formation of coralyne.

hydrolysis of Ia in 4% human plasma markedly decreased in the presence of 0.42% HSA.

However, comparisons of the rate constants for hydrolysis of Ia (Table 6) at pH 7.4 in the presence and absence of 0.42% HSA indicates that HSA itself exhibits some esterase-like activity towards Ia. Related observations have been made previously by Kurono et al. (1979) and Ozeki et al. (1980) who reported HSA catalyzed hydrolysis of several straight and branched chain esters of *p*-nitrophenol. Other workers found that the binding of these ester homologs to the reactive sites of HSA was markedly affected by the substituents on the acyl group (Okeda and Horiguchi, 1980). While the results in Table 6 are mixed and difficult to interpret, they are consistent with interactions between albumin and enzymes resulting in a decreased opportunity for reaction between substrate and more active hydrolytic sites of the enzyme-containing protein. However, other explanations cannot be ruled out on the basis of this limited study.

In the case of the cyclization of II, as shown in Fig. 3, the magnitude of the observed rate constant decreased in what appeared to be a asymptotic fashion. An approximately 20% decrease in k_c occurred when the plasma concentration was increased from zero (aqueous buffer) to 10%. These results can be explained on the basis of protein binding of the 6'-AP in plasma, where the bound form of II cyclizes at a slower rate than the unbound form. As the plasma (protein) concentration increases, the fraction bound increases, and the observed value of k_c decreases asymptotically as the bound fraction approaches unity. Although the changes in k_{obs} over the range of plasma concentration studied is small, it might be anticipated that a substantial slowing of the cyclization reaction might occur in whole plasma and blood, thereby significantly extending the time during which II may exist in vivo. Such an occurrence may then allow for greater in vivo (intracellular) distribution of both II and III than would be expected on the basis of the stability of II in simple aqueous buffer at pH 7.4.

Hydrolysis in tissue supernatant

As had been done earlier (Patel and Repta, 1981) for α-acyloxystyrenes, the rates of hydrolysis of the 6'-AP enol esters by soluble components of rat liver and brain homogenate was studied. A comparison of the tissue homogenate hydrolysis data in

TABLE 7

RATE CONSTANTS AND HALF-LIVES FOR THE FORMATION OF CORALYNE FROM 6′-ACETYLPAPAVERIN (6′-AP) AND ITS ENOL ESTERS IN 105,000×g RAT LIVER [a] and BRAIN [b] SUPERNATANT AT 37°C

Compound	Liver supernatant		Brain supernatant	
	$k \times 10^3$ (min^{-1})	$t_{1/2}$ (min)	$k \times 10^3$ (min^{-1})	$t_{1/2}$ (min)
6′-AP	533	1.3	533	1.3
6′-AP enol acetate	23.9	29	14.8	47
6′-AP enol isobutyrate	22.4	31	6.36	109
6′-AP enol pivalate	3.85	180	– [c]	– [c]

[a] 7.8 mg wet tissue/ml of pH 7.4, isotonic Sorenson's phosphate buffer.
[b] 50 mg wet tissue/ml of pH 7.4, isotonic Sorenson's phosphate buffer.
[c] Hydrolysis not complete in 60 h.

Table 7 with those data obtained in aqueous buffers (Table 3) demonstrates significant catalysis by the soluble enzymes in both the rat brain and liver homogenates. Ic, which contains the considerably sterically hindered pivaloyl group, was the poorest substrate for these esterases as was found for α-pivaloyloxystyrene in our earlier work (Patel and Repta, 1981). The hydrolysis of Ic in rat brain supernatant was incomplete at 60 h (Table 7). The fact that hydrolysis was slowly occurring was confirmed by the changes in the UV spectrum of the hydrolysis mixture which were similar to those reported by Cho et al. (1975) for the cyclization of 6′-AP to the coralyne ion.

There was a significant difference in the rates of hydrolysis of the acetate and isobutyrate enol esters of 6′-AP in the brain homogenate, but no such difference was observed in the liver supernatant (Table 7) at the concentration studied. The quantitative interpretation of these results is difficult because of the limited amount of data and the complexity of the rat tissue supernatants. However, it may be concluded that the esterases in brain and liver supernatants exhibit differing specificity but still catalyze the hydrolysis of all of the 6′-AP enol esters studied.

Conclusions

This study demonstrated that the acetate, isobutyrate and pivalate enol esters of 6′-acetylpapaverin possessed adequate stability in aqueous buffers to be useful as potential prodrug forms of the coralyne ion. The reversion of these esters to 6′-AP, which cyclizes rapidly to coralyne, is catalyzed by esterases present in rat and human plasma and rat liver and brain tissue supernatants. The rates of hydrolysis of pivalate and isobutyrate and acetate and enol esters in aqueous buffers and biological media differed appreciably and appeared to correlate with increasing steric hindrance around the carbonyl group. The data obtained from these in vitro studies further suggest that the enol esters of 6′-AP may serve as useful prodrug forms for enhancing delivery of the experimental antitumor agent, coralyne, to brain

tissue. Results of in vivo rat studies on the use of Ia, Ib and Ic as delivery forms of coralyne will be presented in a subsequent report.

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