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ENOL ESTERS AS POTENTIAL PRODRUGS I. STABILITY AND ENZYME-MEDIATED HYDROLYSIS OF α-ACETOXYSTYRENE

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SUMMARY

The stability of α-acetoxystyrene, a model enol ester, was evaluated in aqueous buffered solvents and in human and rat plasma and rat tissue homogenates. The compound was found to be a good substrate for endogenous esterases exhibiting a half-life for hydrolysis in human plasma of <3 min while in phosphate buffer at pH 7.4 and 25°C the calculated $t_{1/2}$ was ~180 h. These results suggest that enol esters may be useful as prodrugs of agents containing an enolizable carbonyl group.

INTRODUCTION

There are numerous drugs containing enolizable carbonyl groups as their most prominent functional group and which exhibit delivery problems (usually due to low aqueous solubility). Examples of such a drug include diphenadione and a number of related anticoagulants and phenylbutazone and closely related anti-inflammatory agents.

Although all of these agents may undergo enolization, the keto–enol equilibrium usually lies far in favor of the keto form (Scheme 1). However, under proper conditions the enol form can be trapped by alkylation or acylation of the hydroxyl (enol) group. Such enol ethers and esters upon hydrolysis liberate the free enol which reverts to the keto form nearly instantaneously. Thus it seems that enolizable carbonyl groups in certain problem drugs might serve as sites which could be derivatized to yield potentially useful prodrugs.

In the identification and evaluation of any potential prodrug, the in vitro and in vivo stability of the prodrug must be considered. The prospective prodrug must exhibit ade-

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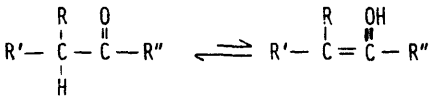
SCHEME 1



KETO-FORM ENOL-FORM

quate in vitro stability such that it can be incorporated into the desired dosage form, but upon administration it must revert to the parent compound quickly and completely. In the case of normal esters, the reversion is triggered by endogenous esterases (Repta et al., 1975; Junge and Krisch, 1975).

Pinson (1959) and Izquierdo and Gomis (1971) have reported the preparation of several enol esters of phenylbutazone (a 1,3-diketone and also a carbon acid). There are also several reports of the synthesis of enol esters of keto steroids (Liston, 1966; Hirschmann and Wendler, 1953; Vanderhaeghe et al., 1952). Schöpf (1929) has suggested that dihydrocodeinone enol acetate may be 'applicable in therapy'. This enol ester is presently marketed under the proprietary name Acedicon (Blacow, 1972). While it seems reasonable that enol esters should be substrates for the various endogenous esterases, no data supporting such an assumption have appeared in the literature. Furthermore, there appear to be no reports comparing the in vitro and in vivo stability of enol esters.

The work reported here utilized α-acetoxystyrene as a model enol ester for assessing the relative stability of enol esters in buffered aqueous solutions and in plasma and other tissue homogenates. Although the hydrolysis of α-acetoxystyrene has been studied in strongly acidic solution (Noyce and Pollack, 1969) and in alkaline solutions (Novak and London, 1977), there appears to have been little or nothing done in the neutral range (pH 3–10) or in biological tissue homogenates or fluids such as plasma. Acetophenone was chosen as a model for the ketone-containing portion of the experimental antitumor agent, 6′-Acetylpapaverine (Cho et al., 1975).

MATERIALS AND METHODS

α-Acetoxystyrene was synthesized by the method of Noyce and Pollack (1969). The boiling point, elemental analysis data and NMR spectrum were in excellent agreement with the published data. The rates of first-order hydrolysis were monitored by following the changes in absorbance at 278 nm either directly (as in the buffered aqueous solutions) or by first extracting the human plasma samples with chloroform. A stock solution of α-acetoxystyrene was prepared in acetonitrile and an appropriate volume was mixed with the aqueous buffer or plasma for the kinetic studies. The final concentration of acetonitrile was always less than 0.5% (v/v). Pooled human plasma from a blood bank was used. Male Sprague–Dawley rats weighing 250–300 g were used to obtain plasma and other tissue homogenates. The tissues were homogenized at 0–5°C in a glass–teflon homogenizer and centrifuged at 10°C at 105,000 × *g* for 90 min to obtain the rat liver supernatants. Sorenson's isotonic phosphate buffer (pH 7.4) was used to dilute plasma and prepare tissue homogenates.

RESULTS AND DISCUSSION

The rates of hydrolysis of α-acetoxystyrene in pH 7.4, 0.025 M phosphate buffer at several temperatures are shown in Table 1. An Arrhenius plot of the data in Table 1 gave a value of 19.2 ± 0.3 kcal/mol for the apparent energy of activation. An Eyring plot of the same data yields a value of 18.8 ± 0.4 kcal/mol and −23.21 e.u. for the apparent enthalpy and entropy of activation, respectively. The linear regression coefficient for both of these plots was greater than 0.999. The values for the activation parameters for the hydrolysis of α-acetoxystyrene at pH 7.4 are similar to those reported for the hydrolysis of certain esters and amides such as aspirin (Garrett, 1957), atropine (Zvirblis et al., 1956) and N-acetyl-*p*-aminophenol (Koshy and Lach, 1961) in neutral aqueous media. From the data in Table 1 the half-life for the hydrolysis of α-acetoxystyrene in water at 25°C and pH 7.4 was calculated to be a out 180 h. The rate of hydrolysis was independent of the total phosphate concentration at pH 7.4.

The kinetics of the hydrolysis of α-acetoxystyrene was also studied in various concentrations of human plasma at 37°C (Table 2). It is quite evident from the data in Table 2 that plasma esterases significantly catalyze the hydrolysis of α-acetoxystyrene in vitro and it is to be anticipated that similar results would be obtained in vivo.

α-Acetoxystyrene was also a good substrate for the soluble esterases of rat plasma and the 105,000 × *g* rat liver supernatant. Rat plasma had significantly higher esterase activity (towards α-acetoxystyrene as substrate) than did human plasma. For example, the half-

TABLE 1

RATE CONSTANTS AND HALF-LIVES FOR THE HYDROLYSIS OF α-ACETOXYSTYRENE IN PHOSPHATE BUFFER (pH 7.4, 0.025 M) AT VARIOUS TEMPERATURES

Temp. (°C)	Half-life $t_{1/2}$ (h)	Rate constant $k_{obs} \times 10^2$ (h^{-1})
37	56.8	1.2
50	16.8	4.1
58	7.8	8.9
66	4.2	16.6

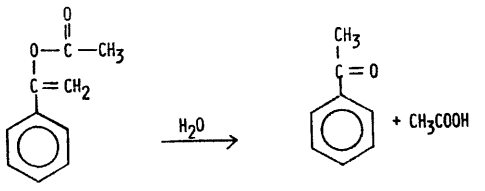
TABLE 2

HALF-LIVES FOR THE HYDROLYSIS OF α-ACETOXYSTYRENE IN HUMAN AND RAT PLASMA AND RAT LIVER SUPERNATANT [a]

Tissue	Concentration	Half-live (sec)
Human plasma	100% (v/v)	160
Human plasma	50% (v/v)	285
Rat plasma	1% (v/v)	115
Rat liver	0.2% (w/v)	344

[a] See experimental.

SCHEME 2



life for the hydrolysis of α-acetoxystyrene in 1% rat plasma (in pH 7.4, isotonic Sorenson's phosphate buffer) was 115 sec as compared to 160 sec in 100% human plasma (Table 2).

CONCLUSIONS

As a result of these studies, it appears that enol esters such as α-acetoxystyrene are relatively stable in aqueous solutions at pH 7.4, but they do revert to the parent ketone fairly rapidly and completely in the presence of the esterases of human and rat plasma and tissue homogenates (Scheme 2). Therefore enol-esters may be useful prodrug derivatives of enolizable ketones.

Studies are underway to determine the effect of different acyl groups on the in vitro and in vivo stability of a series of α-acyloxystyrenes in simple buffered aqueous solutions and in plasma and tissue homogenates.

ACKNOWLEDGEMENT

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