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Drug latention of terbutaline

In vitro serum esterase catalyzed hydrolysis of a series of acyl-substituted mono- and diesters of terbutaline

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SUMMARY The *in vitro* serum esterase catalyzed hydrolysis of a series of differently acyl-substituted diesters of terbutaline, i.e. 1-(3',5'-diacyloxyphenyl)-2-*t*-butylaminoethanols, together with some of their corresponding monoesters, have been studied in diluted serum from man, cat and dog. The kinetic behaviour of the esters in human serum support a mechanism for the esterase catalyzed reaction where acylation of the enzyme is rate-limiting. It has also been found that the esterase catalyzed hydrolysis of the terbutaline esters is sensitive to steric hindrance, i.e. increased branching in the acyl moiety of the esters lower the rate of hydrolysis.

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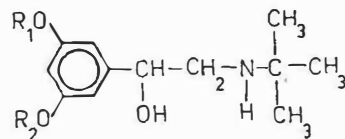
Generally, phenolic compounds are metabolically conjugated to sulphuric acid esters and/or glucuronides during absorption from the gastrointestinal tract [1]. The major site for the conjugation reaction seems in many instances to be the liver, but the gut wall, too, may play an important role in these reactions [2]. As the conjugates of biologically active compounds usually are both considerably less active than the parent compounds and also more readily excreted, conjugation may *a priori* be regarded as an unwanted reaction by the body metabolism on the drug molecule.

The bronchodilator drug, terbutaline (Bricanyl®) [3] is conjugated by sulphuric acid to an extent of 85 % of the dose absorbed after oral administration, while about 60 % of the doses given intravenously or subcutaneously are excreted as unconjugated drug [4]. It has not yet been established whether the major part of the conjugate metabolite is formed in the gut wall during absorption or is the result of a first-pass metabolism in the liver.

As the phenolic OH groups in terbutaline are sites for attack by the conjugating enzymes, as well as being a contributing factor to the high hydrophilicity of the molecule, a blocking of the OH groups by a biologically displaceable functional group, i.e. drug latentation [5], seemed to be an attractive way of overcoming or reducing conjugation during absorption, and at the same time of increasing the lipophilicity of the drug, making it more easily absorbed from the gastrointestinal tract.

The most suitable blocking group to be used in this case seemed to be the acyl group since it can be removed *in vivo* by esterases. Furthermore, since the esterase catalyzed hydrolyses of phenyl acetates have been found to display a normal steric order of reactivity [6], this may give us the opportunity of preparing esters with different biological stability by merely varying the steric bulkiness in the acyl moiety. This is important because the proper biological stability of the ester constitutes a prerequisite if a high concentration of the parent drug is to be brought into the general circulation *via* an efficient first-pass metabolism.

With this in mind, a series of acyl substituted mono- and diesters of terbutaline has been synthesized [3b] and the present paper reports the *in vitro* hydrolysis in sera from man, cat and dog of some of the esters prepared.

KWD 2037: $R_1 = R_2 = \text{CH}_3\text{-CO}$ KWD 2179: $R_1 = \text{H}, R_2 = (\text{CH}_2)_2\text{CH-CO}$ KWD 2074: $R_1 = \text{H}, R_2 = \text{CH}_3\text{-CO}$ KWD 2170: $R_1 = R_2 = \text{CH}_3\text{CH}_2(\text{CH}_2)_3\text{CH-CO}$ KWD 2057: $R_1 = R_2 = \text{CH}_3\text{CH}_2\text{-CO}$ KWD 2085: $R_1 = R_2 = (\text{CH}_3)_3\text{C-CO}$ KWD 2058: $R_1 = R_2 = (\text{CH}_3)_2\text{CH-CO}$

Experimental

Materials

Blood was obtained from healthy volunteers, cats and beagle dogs. Serum, free from visible hemolysis, was prepared and stored at 0–4°C.

All chemicals used were of analytical grade and satisfactory analyses were obtained for all the esters investigated.

Kinetic measurements

The appearance of monoester and terbutaline, respectively, were followed in a Zeiss DMR 21 recording spectrophotometer, equipped with a thermostated cell compartment maintaining constant temperature, $37 \pm 0.1^\circ\text{C}$ for human serum and $38 \pm 0.1^\circ\text{C}$ for animal serum, during the kinetic runs. For each specie, all rate constants are obtained from experiments performed with the same batch of serum, and the constancy of the catalytic activity of the serum was regularly checked with KWD 2058. Into the reaction cuvette containing 2000 μl of a stock solution of serum diluted 1:50 in 0.067 M phosphate buffer pH 7.4, was injected a constant volume of 200 μl freshly made aqueous solution of the esters. In the reference cuvette the ester solution was replaced by plain water. Each substrate was investigated at three concentrations ranging from $5 \cdot 10^{-5}$ to $5 \cdot 10^{-4}$ M, in duplicate. The hydrolysis of the diesters to the corresponding monoesters was measured at the isosbestic point for terbutaline and the monoester, i.e. 277 nm,

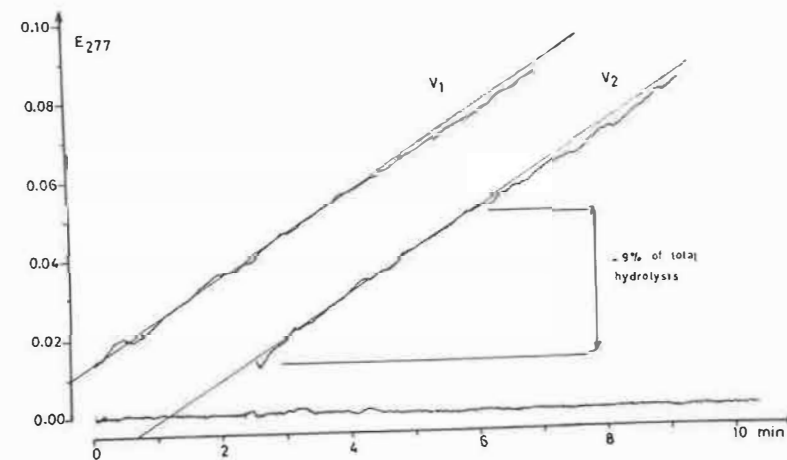


Fig. 1. Esterase-catalyzed hydrolysis of KWD 2058 in human serum.

Duplicate runs in serum diluted 1:55 in 0.061 M phosphate buffer pH 7.4 at 37.0°C . $[s] = 2.5 \cdot 10^{-4}$ M.
 $v_1 = v_2$ = initial rates.

$\Delta \varepsilon = 1.8 \cdot 10^3 M^{-1} \text{ cm}^{-1}$. The hydrolysis of the monoesters to terbutaline was followed at 286 nm where $\Delta \varepsilon = 5.1 \cdot 10^2 M^{-1} \text{ cm}^{-1}$. For all substrate concentrations, the expanded measuring range, 0—0.1, of the spectrophotometer was used to follow the rise in absorption. Initial rates were obtained from the curve representing the first 5—10 % of the reaction (see Fig. 1) except for the lowest concentration of monoesters where a somewhat higher degree of reaction had to be followed. Appropriate corrections were made for the spontaneous hydrolysis of the esters.

Treatment of data

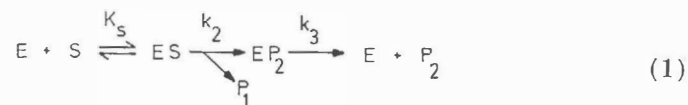
The initial rates were plotted according to the methods given by Eadie (v vs. $\frac{v}{[S]}$) [7] and Lineweaver-Burk ($\frac{1}{v}$ vs. $\frac{1}{[S]}$ and $\frac{[S]}{v}$ vs. $[S]$) [8]. Both methods gave similar results without any significant differences in the parameters or their deviations [9]. The values given in Tables 1 and 2 are obtained from the Eadie plots. (See also Fig. 2 for an example of an Eadie plot). The constants K_m , V_m , $\frac{V_m}{K_m}$ and standard deviations were estimated on a HP-calculator 9810 by linear regression using an unweighted least-squares method.

Results and discussion

Evidence that serum esterases really catalyze the hydrolysis of the terbutaline esters is found in the increase in rates of hydrolysis observed when diluted serum (1:50) is added to a buffered water solution of the esters. The esterase catalyzed reaction under such conditions is thus found to cause a rate acceleration of more than 10^3 , when compared to the non-enzymatic hydrolysis in water [10]. Addition of physostigmine ($10^{-5} M$) to the sample was also found to considerably inhibit serum esterase catalyzed hydrolysis of the ester of terbutaline ($5 \cdot 10^{-4} M$).

Moreover, zymograms (polyacrylamide gel electrophoresis) from human serum reveal only one esterase component that hydrolyses the terbutaline esters, while three esterase components are found in cat serum [11]. Since cat serum contains more than one esterase component, and no data is available for dog serum, the following discussion will mainly be concerned with hydrolysis in human serum.

Generally, esterases operate according to a three-step mechanism [12], Eq. (1)



where E is the enzyme, S the substrate, ES the enzyme-substrate complex, EP₂ the acylated enzyme and P₁ and P₂ the products, i.e. alcohol (phenol) and fatty acid, respectively.

This model, Eq. (1), may *a priori* also be used to describe the serum esterase catalyzed hydrolysis of the terbutaline esters.

The hydrolysis experiments were followed in UV by monitoring the release of the phenolic compounds. Diluted serum had to be used as protein absorption in UV coincides at the wavelengths where terbutaline and its monoesters absorb. Under conditions when $[S]_0 \gg [E]_0$, and $[S]_0 = \text{constant}$, a linear relation between initial rates and degree of dilution of the serum was observed, as predicted by the Michaelis-Menten equation [13], Eq. (2).

Table 1

Kinetic parameters for the human serum esterase-catalyzed hydrolysis of acyl-substituted terbutaline esters^a in 0.061 M phosphate buffer pH 7.4 and serum diluted 1:55 at 37.0°C.

Compound code No.	V_m $M \text{ sec}^{-1} \cdot 10^8$	$(V_m)_{rel}$	K_m $M \cdot 10^5$	$\frac{V_m}{K_m}$ $\text{sec}^{-1} \cdot 10^3$	$\left(\frac{V_m}{K_m}\right)_{rel}$
KWD 2037 $R_1 = R_2 = CH_3CO$	71.1 ± 3.0	7.0	7.1 ± 0.7	10.0 ± 0.7	2.3
KWD 2074 $R_1 = H; R_2 = CH_3CO$	98.6 ± 1.9	9.8	4.9 ± 0.3	20.1 ± 0.8	4.7
KWD 2057 $R_1 = R_2 = CH_3CH_2CO$	73.6 ± 1.0	7.3	5.2 ± 0.2	14.0 ± 0.5	3.3
KWD 2058 $R_1 = R_2 = (CH_3)_2CHCO$	10.1 ± 0.1	1.0	2.3 ± 0.1	4.4 ± 0.2	1.0
KWD 2179 $R_1 = H; R_2 = (CH_3)_2CHCO$	89.2 ± 1.0	8.8	2.3 ± 0.1	38.1 ± 1.9	8.9
KWD 2085 $R_1 = R_2 = (CH_3)_3CCO$	0.17 ± 0.02	0.02	4.8 ± 1.7	0.04 ± 0.01	0.008
KWD 2170 $R_1 = R_2 = CH_3CH_2(CH_2)_3CH$	1.6 ± 0.1	0.16	2.6 ± 0.7	0.6 ± 0.1	0.15

^a The rate constants for the diesters KWD 2037, 2057, 2058, 2085 and 2170 refer to hydrolysis of diester to monoester.

$$v_o = \frac{\frac{k_2 \cdot k_3}{k_2 + k_3} [E]_o [S]_o}{[S]_o + \frac{K_s \cdot k_2}{k_2 + k_3}} = \frac{V_m \cdot [S]_o}{[S]_o + K_m} \quad (2)$$

where

v_o = initial rate

$[S]_o$ = initial substrate conc.

$[E]_o$ = total enzyme conc.

V_m = maximal rate

K_m = the Michaelis-Menten constant

Eq. (2) was applied for calculation of rate constants by treating the values obtained for initial rates at different substrate concentrations according to the method given by Eadie. From the straight lines of these plots the values of the constants K_m , V_m and $\frac{V_m}{K_m}$ could be obtained, and in Tables 1 and 2 these constants are given for the hydrolysis of the esters in human, cat and dog serum. Fig. 2 shows a representative example of an Eadie plot for the hydrolysis of one of the esters, KWD 2058, in human serum.

Although our data do not permit any detailed kinetic description of the mechanism of hydrolysis of the terbutaline esters, see Eq. (1), there

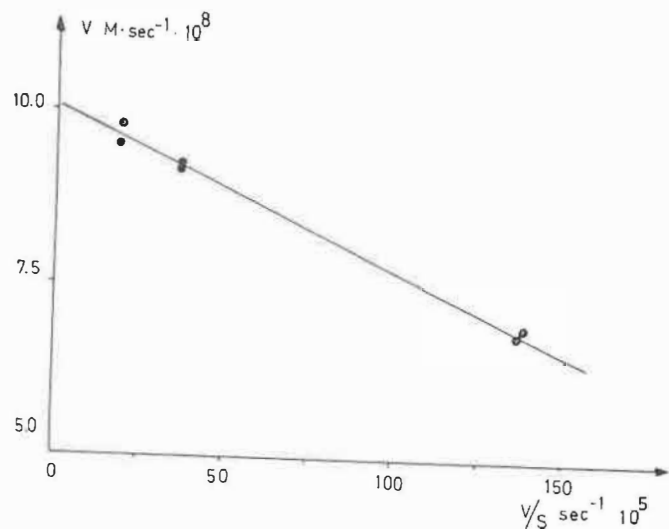


Fig. 2. Eadie plot for the esterase-catalyzed hydrolysis of KWD 2058 in human serum diluted 1:55 in 0.061 M phosphate buffer pH 7.4, at 37.0°C.

are, in fact, certain indications in support of acylation, i.e. k_2 in Eq. (1), being rate limiting in the reaction. This means that Eq. (1) may be reduced into Eq. (3).



Support for this assumption is found in the fact that diesters KWD 2037 and KWD 2058 hydrolyze to their corresponding monoesters, KWD 2074 and KWD 2179, at a slower rate than these monoesters hydrolyze

Table 2

Kinetic parameters for the cat and dog serum esterase-catalyzed hydrolysis of acyl-substituted terbutaline esters* in 0.061 M phosphate buffer pH 7.4 and serum diluted 1:55 at 38.0°C.

Compound code No.	V_m $M \text{ sec}^{-1} \cdot 10^8$	$(V_m)_{rel}$	K_m $M \cdot 10^5$	$\frac{V_m}{K_m}$ $\text{sec}^{-1} \cdot 10^3$	$\left(\frac{V_m}{K_m}\right)_{rel}$
<i>Cat</i>					
KWD 2037 $R_1 = R_2 = \text{CH}_3\text{CO}$	37.9 ± 0.6	2.6	7.1 ± 0.3	5.4 ± 0.1	2.3
KWD 2074 $R_1 = \text{H}; R_2 = \text{CH}_3\text{CO}$	15.4 ± 0.5	1.1	13.2 ± 0.8	1.17 ± 0.03	0.5
KWD 2057 $R_1 = R_2 = \text{CH}_3\text{CH}_2\text{CO}$	27.0 ± 0.5	1.9	2.4 ± 0.2	11.0 ± 0.7	4.8
KWD 2058 $R_1 = R_2 = (\text{CH}_3)_2\text{CHCO}$	14.3 ± 0.3	1.0	6.2 ± 0.3	2.3 ± 0.1	1.0
KWD 2179 $R_1 = \text{H}; R_2 = (\text{CH}_3)_2\text{CHCO}$	7.3 ± 0.2	0.5	2.8 ± 0.3	2.6 ± 0.2	1.1
KWD 2085 $R_1 = R_2 = (\text{CH}_3)_3\text{CCO}$	0.30 ± 0.02	0.02	2.3 ± 0.7	0.13 ± 0.03	0.06
KWD 2170 $R_1 = R_2 = \text{CH}_3\text{CH}_2(\text{CH}_3)\text{CH}$	5.4 ± 0.1	0.4	2.3 ± 0.2	2.3 ± 0.1	1.0
<i>Dog</i>					
KWD 2058 $R_1 = R_2 = (\text{CH}_3)_2\text{CHCO}$	37.4 ± 1.8	1.0	59.4 ± 3.5	0.63 ± 0.01	1.0
KWD 2179 $R_1 = \text{H}; R_2 = (\text{CH}_3)_2\text{CHCO}$	27.0 ± 1.3	0.7	10.4 ± 1.0	2.6 ± 0.1	4.1

* The rate constants for the diesters 2037, 2057, 2058, 2085 and 2170 refer to hydrolysis of diester to monoester.

to terbutaline. As can be seen in Table 1, the values of V_m for KWD 2058 and KWD 2179 differ by a factor 9. For KWD 2037 and KWD 2074 a similar but less pronounced result is obtained, i.e. 1.4. This would not be the case if deacylation were rate limiting, i.e. $k_2 \gg k_3$ in Eq. (1), because the diester-monoester couple 2058/2179 (or 2037/2074) would form the same acyl enzyme intermediates which then would hydrolyze with the same maximal rates, i.e. $V_m \simeq k_3 \cdot [E]_0$.

Since the different values of V_m calculated for the two diester-/monoester couples cannot describe the rates of hydrolysis of a common intermediate, we instead suggest that the maximal rates may be better represented by $V_m \simeq k_2 \cdot [E]_0$, i.e. with acylation being rate-determining. Similar findings have also been made by Hofstee in his studies on the esterase catalyzed hydrolysis of fatty acid esters of *o*- and *m*-hydroxy-substituted benzoic acids [14].

It is known that with increasingly poorer leaving groups, the rate of acylation will become successively slower, i.e. a change in the nature of the rate determining step will take place [15]. This may be the reason for our substrates, as well as those of Hofstee, having the acylation step rate limiting, because these substrates have relatively poor leaving groups when compared to the *p*-nitrophenyl acetates for which usually $k_3 \ll k_2$.

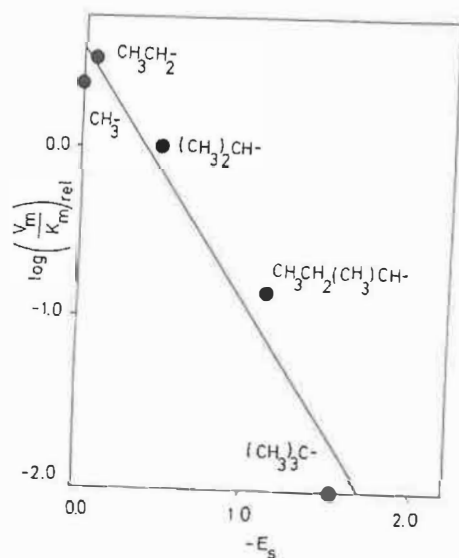


Fig. 3

Plot of $\log \left(\frac{V_m}{K_m} \right)_{rel}$ vs. E_s for the human serum esterase catalyzed hydrolysis of some terbutaline diesters.

Values from Table 1.
Slope value 1.53 ± 0.20 .

Table 3

Relative rate ratios for the acylation of α -chymotrypsin by esters of *p*-nitrophenol and for the serum esterase catalyzed hydrolysis of some terbutaline esters.

Ester	$\left(\frac{k_2}{K_m} \right)_{rel}^a$	$\left(\frac{k_{cat}}{K_m} \right)_{rel}^b$	$\left(\frac{V_m}{K_m} \right)_{rel}^c$
Acetate	1.0	1.0	1.0
Propionate	2.1	—	1.4
Isobutyrate	1.2	0.59	0.4
Pivalate	0.15	0.04	0.004

^a *p*-Nitrophenylesters. Ref. [6].

^b *p*-Nitrophenylesters. Ref. [17].

^c Terbutaline esters. This study.

From the values of V_m , $\frac{V_m}{K_m}$ and $\left(\frac{V_m}{K_m} \right)_{rel}$ in Table 1, it is evident that increased branching of the alkyl group in the acyl portion of the esters, affects the rate of the esterase catalyzed hydrolysis in the proper direction. This is further demonstrated in Fig. 3, where $\log \left(\frac{V_m}{K_m} \right)_{rel}$ is plotted vs. the Taft steric substituent constant, E_s [16]. These data also correlate well with similar data obtained from acylation of α -chymotrypsin with differently acyl substituted *p*-nitrophenylesters [6, 17] (Table 3). Variation of the alkyl substituent in the acyl group of the esters does not, however, seem to have any significant influence on the values of K_m .

In human serum, the monoesters KWD 2074 and KWD 2179 were found to hydrolyze faster than the corresponding diesters KWD 2037 and KWD 2058. Also, in a preliminary experiment, no build-up of the monoester intermediate was found when the hydrolysis of KWD 2058 in human serum was monitored at pH 5.9 where it was possible to obtain an isosbestic point for KWD 2058 and terbutaline, ($\lambda = 250$ nm, 0.061 M phosphate buffer). In cat serum, however, where the diester KWD 2058 apparently hydrolyses faster than its monoester KWD 2179, it was possible, by using the same technique, to get a qualitative build-up and breakdown of the monoester (Fig. 4). Although the experimental conditions were rather unfavourable, the rates estimated for the build-up and breakdown reactions of the monoester were found to agree rather well with those obtained separately at pH 5.9 for the hydrolysis of the di- and monoester, respectively, in cat serum.

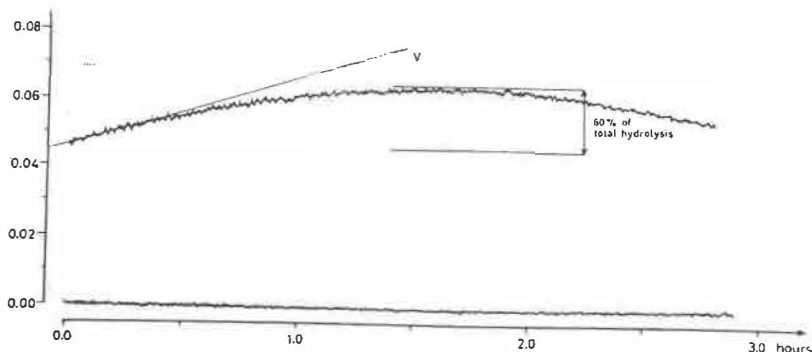


Fig. 4. Qualitative build-up and break-down of the monoester (KWD 2179) in the esterase-catalyzed hydrolysis of KWD 2058 in cat serum diluted 1:55 in 0.061 M phosphate buffer pH 5.9 at 38.0°C.

v = rate of formation of KWD 2179.

The observation that the diesters hydrolyze slower than the monoesters in the esterase catalyzed reaction is contrary to the findings for the non-enzymatic hydrolysis of these compounds, (0.061 M phosphate buffer, pH 7.4), where the diester KWD 2058 is hydrolyzed twice as fast as the monoester KWD 2179. Steric hindrance during the acylation reaction may be one of the reasons for the faster hydrolysis of the monoesters, and this effect may well be reflected in the ratios

$$\left(\frac{V_m}{K_m}\right)_{\text{monoester}} : \left(\frac{V_m}{K_m}\right)_{\text{diester}} \quad \text{for the acetate- and isobutyrate}$$

esters respectively. The ratio for the acetate esters should then be closest to unity, because the increase in steric hindrance caused by the transformation of a monoacetate into a diacetate will be less than when a mono-isobutyrate is transformed into a diisobutyrate. Such results are also obtained in human serum, where

$$\left(\frac{V_m}{K_m}\right)_{\text{monoester}} : \left(\frac{V_m}{K_m}\right)_{\text{diester}} \quad \text{was found to be 2.0 for the acetates,}$$

while a value of 8.9 is obtained for the isobutyrate.

When the rate constants obtained from cat serum (Table 2) are treated in a similar manner, the ratios 2.2 and 1.1 are obtained for the acetates and isobutyrate respectively, which is contrary to what is found in hu-

man, however, be strongly questioned as cat serum contains at least three esterase components capable of hydrolyzing the terbutaline esters.

As was mentioned in the introduction, the biological half-life of a prodrug is of great importance; e.g. if the prodrug *per se* has interesting pharmacological properties in connection with the parent drug, a prodrug having a relatively long half-life would be beneficial, while a pharmacologically inactive prodrug, or one having unwanted effects, should preferably have a short half-life. If there are complications in form of the parent drug being easily conjugated during the absorption process, the half-life of the prodrug must be chosen so that extensive hydrolysis by esterases in the gut wall and liver will be prevented.

The kinetic data presented in this paper for the serum catalyzed hydrolysis of a series of differently acyl substituted esters of terbutaline, show

that a difference in rates of hydrolysis $\left(\frac{V_m}{K_m}\right)$ of more than 10^2 is found within the series. Since all esters in the series show enhanced oral absorption compared with the parent compound, terbutaline, one of the esters, KWD 2058, having an intermediate rate of hydrolysis, has been selected for further evaluation in animal studies. These results are reported elsewhere [18].

References

1. *Metabolic Conjugation and Metabolic Hydrolysis*, Vol. I, W. H. Fishman Ed. Academic Press 1970, pp. 66 and 239
- 2a. C. F. George, E. W. Blackwell and D. S. Davies. *J. Pharm. Pharmacol.* 26, 265 (1974)
- b. W. H. Barr and S. Riegelman. *J. Pharm. Sci.* 59, 154 (1970)
- c. *Ibid.* p. 164
- 3a. J. Bergman, H. Persson and K. Wetterlin. *Experientia* 25, 899 (1969)
- b. K. Wetterlin and L. Å. Svensson. Swedish Pat. No. 335 359 (1971)
4. H. T. Nilsson, K. Persson and K. Tegnér. *Xenobiotica* 2, 363 (1972)
- 5a. N. J. Harper. *J. Med. Pharm. Chem.* 1, 467 (1959)
- b. L. Å. Svensson. *Drug Latentiation. Studies on Catechol Esters of Certain Diacids*. Diss. Lund 1972.
6. J. B. Milstien and T. H. Fife. *Biochemistry* 8, 623 (1969)
7. G. S. Eadie. *J. Biol. Chem.* 146, 85 (1942)
8. H. Lineweaver and D. Burk. *J. Amer. Chem. Soc.* 56, 658 (1934)
9. D. S. Riggs. *The Mathematical Approach to Physiological Problems*, The Williams and Wilkins Co. Baltimore 1967, p. 276
10. L. Nyberg. AB Draco. Personal communication
11. H. T. Nilsson. AB Draco. Personal communication
12. K. Krisch. »The Enzymes» Vol. V., 3rd edition., P. D. Boyer Ed., p. 60 and U. C. Froede and I. B. Wilson. *Ibid.* p. 95

13. L. Michaelis and M. L. Menten. *Biochem. Z.* 49, 337 (1913)
- 14a. B. H. J. Hofstee. *J. Biol. Chem.* 207, 219 (1954)
 - b. B. H. J. Hofstee. *Biochem. Biophys. Acta* 32, 182 (1959)
15. W. P. Jencks. *Catalysis in Chemistry and Enzymology*. McGraw-Hill, New York, 1969, p. 52
16. R. W. Taft. *Steric Effects in Organic Chemistry*, M. S. Newman, Ed., New York, Wiley, Chapter 13, p. 556
17. A. Dupaix, J.-J. Béchet and C. Roucous. *Biochem.* 12, 2559 (1973)
18. O. A. T. Olsson, C. G. A. Persson, H. Persson and L. Sörenby. *Acta Pharmacol. Toxicol.* 35, 76 (1974)

Received July 5, 1974.

Gas chromatographic determination of meprobamate in plasma after column extraction

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SUMMARY A procedure for the quantitative determination of therapeutic levels of meprobamate in plasma is described. The method makes use of a selective column extraction of the drug and a final analysis by gas chromatography. The conditions of the extraction are discussed as well as the choice of a suitable internal standard. A study of the thermal decomposition of meprobamate in the gas chromatographic injector system is also presented. The recovery of samples containing 5 µg/ml is $99.6 \pm 1.6\%$. 0.2 µg/ml is the lowest concentration which can be determined with acceptable precision.

Quantitative determination of meprobamate in biological material has been carried out by colorimetry [1—3] and gas chromatography [4—12]. Several authors using gas chromatography have described difficulties arising from thermal decomposition of meprobamate [4, 6, 11, 13]. Disturbances from other compounds like caffeine and barbiturates have also been reported [9, 12].

This paper describes a method for the gas chromatographic determination of meprobamate in plasma after extraction by partition chromatography. The extraction conditions are calculated from partition studies, and the selectivity of the method is discussed. The influence of the injector temperature on the decomposition of meprobamate as well as the importance of a suitable internal standard are presented.

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