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Methanesulfonic Acid Derivative of *p,p'*-Diaminodiphenylsulfone. I. Hydrolysis Rate *in Vitro*[1,2]

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Various methanesulfonic acid derivatives of *p,p'*-diaminodiphenylsulfone (Da), which are represented as $NaO_3SHCHN\text{-}C_6H_5\text{-}SO_2\text{-}C_6H_5\text{-}NHCHSO_3Na$ (R, R), were synthesized and their hydrolysis rates *in vitro* were investigated. The two methanesulfonic acid groups were hydrolyzed by steps in consecutive first-order reactions. The first hydrolysis rate was larger than the second rate in any of the derivatives. The electron-donating groups facilitate and the electron-withdrawing groups retard the hydrolysis. When R is $HOCH_2(CHOH)_4$ (promin), phenyl, *p*-chlorophenyl, or *p*-nitrophenyl, the hydrolysis rate was pH-dependent and faster in the acidic region. The general acid catalysis by buffer constituents was studied in detail with promin.

As a biopharmaceutical indication on the retardation of free Da liberation, kinetic induction period and the rate of accumulation *in vitro* were evaluated. Result from promin indicated that the liberation of parental Da *in vivo* is strikingly slow and a large part of therapeutic dose of promin, which is markedly larger than that of parental Da, may possibly be wasted in an ineffective form. The derivatives substituted with Me or Et have fast hydrolysis rate and these may be better in the availability of parenteral Da compared to promin.

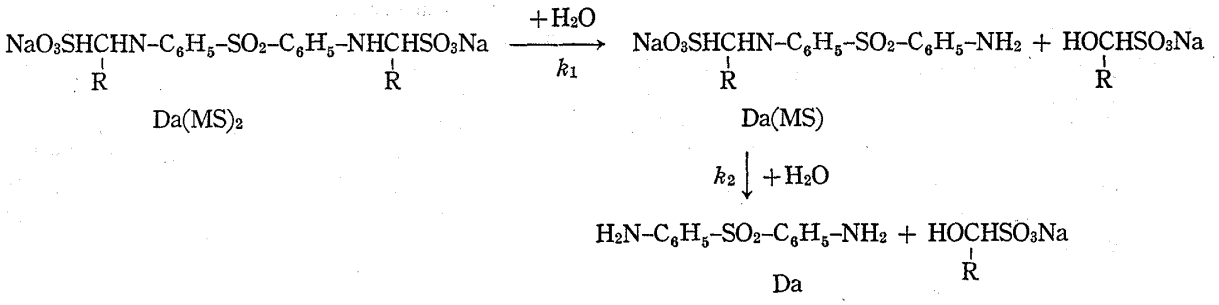
For a series of studies on methanesulfonic acid derivatives (MSD),[4–6] hydrolysis of MSD of *p,p'*-diaminodiphenylsulfone (Da) *in vitro* was investigated. Da has been known to be a strongly active agent for leprosy and tuberculosis, but its toxicity has been a serious problem in the therapy of these diseases. A number of less toxic and highly water-soluble derivatives of Da, such as promin, sodium sulfoxon, and sulfetron, which have appropriate substituent on amino group, were developed and among these, promin is most widely used for leprosy. For the pharmacological activity of these derivatives the hydrolysis of substituent group is indispensable, but no detailed study has been reported for their hydrolysis rate.

In previous studies,[4,5] it has been proved that MSD of aniline substituted with electron-withdrawing group in *para* position has a very small hydrolysis rate. The sulfone group in Da possibly has an unfavorable effect on the hydrolysis. As has been pointed out[5] slow hydrolysis rate causes a substantial loss of the original pharmacological activity. The largely increased dose of promin compared to parental Da (2 g intravenously daily for promin in contrast to 25 mg twice a week and then increasing to a maximum of 100 mg 4 times a week for intact Da[7]) may be an indication of low availability of parental Da. In connection with this anxiety, it is also pointed out that the use of much larger dose of Da in the form of promin

1) Presented at the 92th Annual Meeting of the Pharmaceutical Society of Japan, Osaka, April 1972.
2) This report constitutes Part IV of studies entitled "Methanesulfonic Acid Derivative of Drugs," Part III.[6]
3) Location: *Tanabe-dori, Mizuhoku, Nagoya.*
4) K. Ikeda, K. Miyata, T. Iwata, F. Kawata, and K. Kurome, *Chem. Pharm. Bull.* (Tokyo), **18**, 440 (1970).
5) K. Ikeda, Y. Kurono, and T. Tukamoto, *Chem. Pharm. Bull.* (Tokyo), **20**, 863 (1972).
6) K. Ikeda, Y. Kurono, and T. Tukamoto, *Chem. Pharm. Bull.* (Tokyo), **20**, 1621 (1972).
7) E.E. Smissman, "Textbook of Organic Medicinal and Pharmaceutical Chemistry," 6th ed., ed. by C.O. Wilson, O. Gisvold, and R.F. Doerge, J.B. Lippincot Co., Philadelphia, 1971, p. 298.

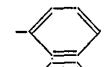
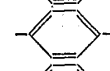
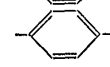
is an economical burden[8] in the developing countries where leprosy is a serious problem.

This hydrolysis can be represented as follows:



The hydrolysis of this kind has been proved to be reversible in previous studies,[4-6] but in the present work, only the forward hydrolysis reaction concerning a change *in vivo* was investigated. As has been revealed, substitution of an appropriate R on methylen group gives a derivative which is hydrolyzed at a predictable rate.[5] Based on these observations, in this report various MSDs have been synthesized and their hydrolysis rate was determined, and also the biopharmaceutical anticipation of these derivatives has been discussed. As the liberation of free Da is delayed owing to the consecutive hydrolysis processes, kinetic induction period and the rate of liberation of free Da were estimated as the indication of the retardation. The abbreviations of the derivatives are shown in Table I.

Table I. Abbreviations

R	Abbreviation	R	Abbreviation
-H	$Da(HS)_2$		$Da(phS)_2$
$-CH_3$	$Da(MeS)_2$		$Da(p\text{-}ClPhS)_2$
$-C_2H_5$	$Da(EtS)_2$		$Da(p\text{-}NO_2PhS)_2$
$-(CHOH)_4CH_2OH$	$Da(GlS)_2$(promin)		

Ph=phenyl

Experimental

Synthesis of MSD of Da——The synthesis followed the methods of Rose,[9] Bauer,[10] and Jain,[11] and the purity of MSD was determined by the colorimetry of parental Da as will be described below.

Determination of Da and Its MSD——Determinations of Da and its MSD were carried out by the colorimetry on the product of diazo coupling. Parental Da and its derivatives are equally colored by the diazo coupling with Tsuda's reagent to the same intensity depending on their molar concentrations. The detailed procedure is essentially the same as described previously.[5] The spectral peak of the color produced is at 530 nm.

Separatory Determination of Parental Da, Da(MS), and $Da(MS)_2$——The separation of $Da(MS)_2$ from Da(MS) and parental Da was carried out by the ion-exchange resin (Amberlite IR-120) column. The separation of Da from Da(MS) and $Da(MS)_2$ was performed by extraction with 1,2-dichloroethane (DCE). Da and Da(MS) are adsorbed on the resin and $Da(MS)_2$ passes through the column quantitatively. By the extraction with DCE the final product of hydrolysis, Da, alone is transfered into DCE. Two ml of the sample solution was taken into a stoppered tube containing 5 ml DCE and the mixture was shaken vigorously for

8) *a*) J. Lowe, *Leprosy Review*, **23**, 4 (1952); *b*) S.G. Browne, "Advances in Pharmacology and Chemotherapy," Vol. 7, ed. by S. Garattini, A. Goldin, F. Hawking, and I.J. Kopkin, Academic Press, Inc., New York and London, 1969, p. 211.
9) L. Rose, Brit. Patent 556 901 (1943) [*C.A.*, **39**, 1881 (1945)].
10) H. Bauer, *J. Am. Chem. Soc.*, **61**, 617 (1939).
11) B.C. Jain, B.H. Iyer, and P.C. Guha, *Science and Culture*, **11**, 568 (1946) [*C.A.*, **40**, 4687 (1946)].

1 min. Then 3 ml DCE phase was pippeted out and added to 5 ml of 3N HCl. After shaking for 1 min, 1 ml of aqueous HCl phase, in which free Da is reextracted, was submitted to colorimetry.

Determination of Hydrolysis Rate——$Da(MS)_2$ solution (about 1.5×10^{-3}M) which was passed through ion exchange column to expel the hydrolysis products, was immediately diluted to about 5×10^{-4}M with buffer solution kept at the reaction temperature. Buffer systems used were M/5 acetate (pH 3.0—5.8) and 1/15M phosphate (pH 6.0—8.0). At appropriate intervals, two aliquot samples were taken and the remaining $Da(MS)_2$, which passes through ion-exchange column, and Da produced, which is extracted into DCE, were determined as above. The concentration of Da(MS) was estimated by the substraction of the amount of $Da(MS)_2$ and Da from that of initial $Da(MS)_2$. The first step hydrolysis rate constant, k_1, was calculated from the half life estimated by the semilogarithmic plots of the remaining $Da(MS)_2$. The second step hydrolysis rate constant, k_2, was determined as follows. From the consecutive first-order reaction rate law, [Da] can be represented as

$$[Da] = [Da(MS)_2]_0\left\{1+\frac{1}{k_1-k_2}(k_2e^{-k_1t}-k_1e^{-k_2t})\right\} \quad (1)$$

Detailed investigation on the Da formation rate, $d[Da]/dt$, revealed that the plot of $\log(d[Da]/dt)$ *vs.* t becomes sufficientry linear with a negative slope at the advanced stage of the reaction. The slope of the plots, which may be assigned to the rate constant of the rate-determining step of the Da formation. was substantially smaller than k_1 value determined previously. This may rationalize the supposition that the second step of the hydrolysis governs the Da formation at the proceeded period of the reaction and e^{-k_1t} term in equation (1) can be neglected at the proceeded period. Hence

$$[Da(MS)_2]_0 - [Da] = [Da(MS)_2]_0\frac{k_1}{k_1-k_2}e^{-k_2t} \quad (2)$$

The slope of the plot of $\log([Da(MS)_2]_0-[Da])$ *vs.* time gives k_2.

Result and Discussion

Hydrolysis Rate in Aqueous Solution

Figure 1 shows a typical consecutive first-order reaction process for $Da(GlS)_2$ at 37° and pH 4.87. Figures 2 and 3 show the pH-profiles of the logarithm of the first step hydrolysis

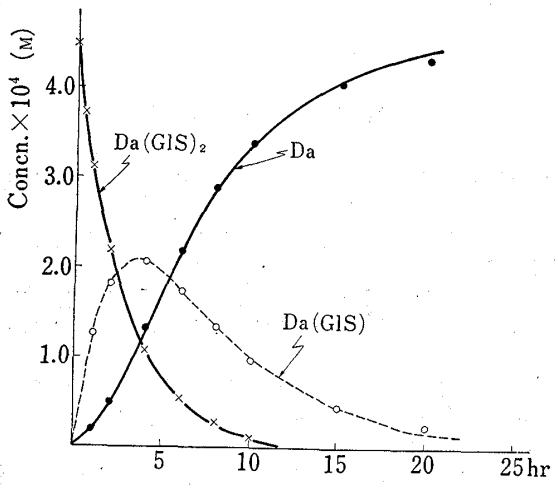


Fig. 1. The Hydrolysis of $Da(GlS)_2$ at 37°, pH 4.87

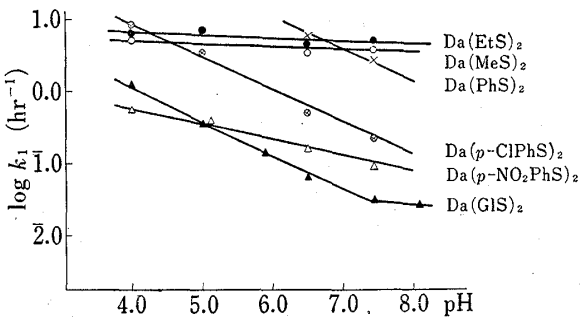


Fig. 2. The pH-profile of the 1st Step Hydrolysis Rate of $Da(MS)_2$ at 37°
Ph=phenyl

rate constant, k_1, and the second step hydrolysis rate constant, k_2, of various MSDs at 37°. The data shown in Fig. 2 and 3 were not corrected for the effects of ionic strength and buffer concentration. As can be seen from these graphs, the pH-profiles for log k_1 and log k_2 have a similar pattern. When R is H, Me, or Et, the hydrolysis rate is almost independent of pH from pH 4.0 to 8.0, which is the same as previous results on the MSDs of substituted aniline[4]

and sulfonsmides.[5] However, when R is Gl, phenyl, *p*-ClPh, or *p*-NO_2Ph, the hydrolysis rate is dependent on pH. The electron-donating substituents, Me, Et, or phenyl, accelerate the hydrolysis compared to the unsubstituted MSD and the substitution of electron-withdrawing group on phenyl, *p*-Cl, or *p*-NO_2. decreases the rate, which shows a good agreement with that obtained previously.[4] The data on Da $(HS)_2$ at 37° were obtained from the extrapolation of Arrhenius plots at a higher temperature (from 60° to 80°), because the hydrolysis rate is too slow. In all cases studied the first step hydrolysis rate constant is larger than the second one.

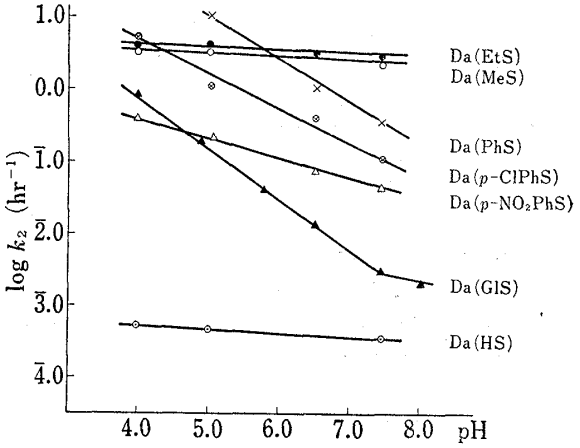


Fig. 3. The pH-profile of the 2nd-Step Hydrolysis Rate of Da$(MS)_2$ at 37°.

Ph=phenyl

Table II summarizes the results of observed value of thermodynamic parameters for the hydrolysis rate. It is apparent from this Table that there are some differences between pH-dependent and pH-independent hydrolyses. Da $(GlS)_2$, Da $(PhS)_2$, Da (*p*-$ClPhS)_2$, and Da (*p*-$NO_2PhS)_2$, which show appreciable pH-dependency of the reaction rate, have substantially larger negative $\Delta S^{\neq}$ values and smaller E_a values in neutral region compared to those in acidic region. On the other hand, Da $(HS)_2$, Da $(MeS)_2$, and Da $(EtS)_2$ show essentially constant $\Delta S^{\neq}$ and E_a values through the whole pH region studied. These may indicate that there is some difference in the reaction mechanism depending on pH, although conclusive argument should be based on further investigation.

Table II. Thermodynamic Parameters of the Hydrolysis Rate, Energy of Activation (E_a), and Activation Entropy ($\Delta S^{\neq}$) (E_a; kcal/mol. $\Delta S^{\neq}$ (37°); e.u.)

k_i	pH	Da-$(HS)_2$		Da-$(MeS)_2$		Da-$(EtS)_2$		Da-$(GlS)_2$		Da-$(PhS)_2$		Da-(*p*-$ClPhS)_2$		Da-(*p*-$NO_2PhS)_2$	
		E_a	$\Delta S^{\neq}$	E_a	$\Delta S^{\neq}$	E_a	$\Delta S^{\neq}$	E_a	$\Delta S^{\neq}$	E_a	$\Delta S^{\neq}$	E_a	$\Delta S^{\neq}$	E_a	$\Delta S^{\neq}$
k_1	3.96	—		—		18.7	3.6	18.4	−0.6	—		16.1	−4.8	15.5	−12.0
	5.08	—		22.4	15.9	—		20.0	2.0	—		14.6	−11.1	15.2	−13.3
	6.53	—		—		16.7	−4.3	—		19.1	4.4	—		13.5	−20.3
	7.44	—		20.4	8.3	13.9*	−13.3	11.9	−29.1	15.8	−7.6	14.3	−17.6	11.3	−28.7
k_2	3.96	24.7	4.1	17.0	−3.4	14.8	−10.1	18.3	−1.7	17.6	3.0	16.4	−4.0	17.0	−7.7
	5.08	25.5	6.3	15.7	−7.5	15.3	−8.7	18.0	−2.1	17.3	−0.1	16.4	−5.3	16.4	−10.8
	6.53	—		—		—		13.1	−26.6	—		15.3	−13.0	12.1	−26.8
	7.44	26.2	7.9	16.4	−6.2	14.2	−13.0	10.7	−37.7	13.5	−19.1	12.7	−24.0	12.1	−27.5

Effect of Buffer Constituents for Da $(GlS)_2$ Hydrolysis

In Figures 4 and 5 the effects of buffer constituents on the hydrolysis of Da $(GlS)_2$ are shown. It is clearly indicated that hydrolysis is the subject to general acid catalysis by buffer constituents. Apparent hydrolysis rate constants in respective buffer, $k_{i,app}$, where i refers to the first or the second step of hydrolysis, can be expressed as

$$(k_{i,app})_{acetate} = k_i^0 + k_{i,AcOH}[AcOH] + k_{i,AcO^-}[AcO^-] \quad (3)$$

$$(k_{i,app})_{phosphate} = k_i^0 + k_{i,H_3PO_4}[H_3PO_4] + k_{i,H_2PO_4^-}[H_2PO_4^-] + k_{i,HPO_4^{2-}}[HPO_4^{2-}] + k_{i,PO_4^{3-}}[PO_4^{3-}] \quad (4)$$

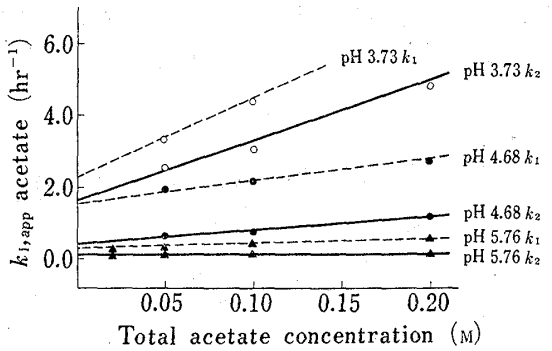


Fig. 4. Dependence of Rate Constants of $Da(GlS)_2$, k_1, on Acetate Buffer Concentrations at Constant pH at 50° and Ionic Strength of 0.173

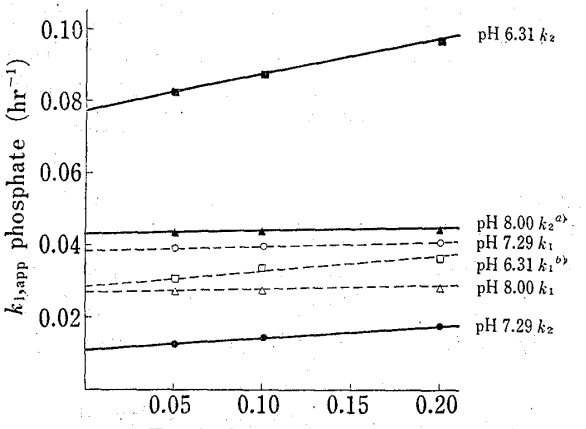


Fig. 5. Dependence of Rate Constants of Da $(GlS)_2$, k_1, on Phosphate Buffer Concentrations at Constant pH at 50° and Ionic Strength of 0.574

a) $k_2 \times 10$ b) $k_1 \times 10^{-1}$

In these equations, k°_i is the rate constant at zero buffer concentration and k_i suffixed with ionic species of buffer constituents is the catalytic constant. In equation (4), k_{i,H_3PO_4} and $k_{i,PO_4^{3-}}$ were not obtainable in the pH region studied. Table III gives the result of the least square calculation[12] of the rate constants from the observed $k_{i,app}$ value assuming equation

TABLE III. Catalytic Rate Constants estimated for the Hydrolysis of $Da(GlS)_2$

1st-step catalytic constant (M^{-1} hr^{-1})	2nd-step catalytic constant (M^{-1} hr^{-1})
$k_{1,AcOH} = 17.3$	$k_{2,AcOH} = 14.0$
$k_{1,AcO^-} = -2.59$	$k_{2,AcO^-} = -2.37$
$k_{1,H_2PO_4^-} = 0.64$	$k_{1,H_2PO_4^-} = 0.98$
$k_{1,HPO_4^{2-}} = -0.24$	$k_{1,HPO_4^{2-}} = -0.42$

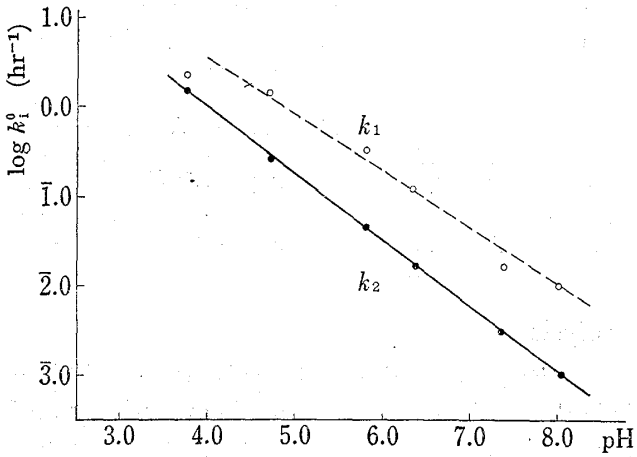


Fig. 6. The pH-depencdence of Rate Constants, k_i°, of $Da(GlS)_2$ at Corrected Ionic Strength of 0.173 and at 50°

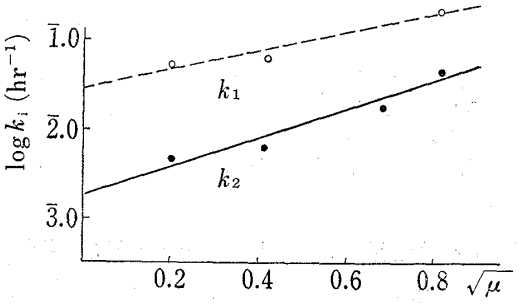


Fig. 7. The Effect of Ionic Strength on Hydrolysis Rates, k_i, for $Da(GlS)_2$ at 50° and pH 7.4

12) S. Numakura, "Sokuteichi Keisanho," Morikita Shuppan, Tokyo, 1969, p. 81.

(3) or (4). As shown, catalytic constants concerning AcO^- and HPO_4^{2-} have a slightly negative value, which may not be attributed to experimental error. Precise explanation on this inhibitory effect is not deducible from the result obtained. Figure 6 is the pH-profile of log k_{ii}^0 at 0.173 ionic strength estimated from the obsevation on the effect of ionic strength. An example of the effect of ionic strength is illustrated in Fig. 7. The slopes of pH-dependency of k_{ii}^0 shown in Fig. 6 were −0.638 and −0.732 for the first and the second step hydrolysis, respectively. Although k_{ii}^0 is not simply proportional to $[H^+]$, it is assumed that hydrogen ion plays a role in the hydrolysis.

Biopharmaceutical Aspect of MSD of Da

As has been seen the release of free Da from MSD is remarkably slow. Taking account of the remarkably slow rate of Da liberation from Da $(GlS)_2$, further studies on the availability of parenteral Da from the clinical dose of this derivative for leprosy are necessary. Refering to the substantially larger dose of this derivative in spite of the high toxicity of parental Da, it is suspected that a larger amount of Da is excreted in the water-soluble form of MSD. The low toxicity which is praised as a merit of this derivative may be a reverse side of the low availability of parental Da. For these problems biopharmaceutical studies are now being carried out in our laboratory and the result will be reported later.

From the results of kinetic study on hydrolysis, the retardation of Da release may be evaluated kinetically from the induction period and the rate of accumulation of free Da. The appearence of Da indicates a sigmoid curve as shown in Fig. 1. The rate of appearence is rationally expressed by the maximum value of $d[Da]/dt$ which occurs at inflection point of [Da] curve (equation (1)). The time of inflection, t_{infl}, is expressed as

$$t_{infl} = \frac{\ln \frac{k_1}{k_2}}{k_1 - k_2} \tag{5}$$

where Da (MS) curve also attains the maximum. The value of $d[Da]/dt$ at t_{infl}, which is represented as $(d[Da]/dt)_{infl}$, is as follows:

$$\left(\frac{d[Da]}{dt}\right)_{infl} = [Da(MS)_2]_0 \frac{k_2}{M} \tag{6}$$

where $M = (k_1/k_2)^{k_2/(k_1-k_2)}$. For practical purpose, it may be appropriate to represent the maximum rate in the unit of percent/hour. The intercept to abscissa of the tangential line at t_{infl} may be used to indicate the induction period, τ, which is expressed as

$$\tau = \frac{1}{k_2}\left(2.303 \log M - M + 1 + \frac{k_1}{k_2}\right) \tag{7}$$

Table IV shows the result of calculation on indications for the retardation of Da liberation from various MSDs. Figure 8 shows the percentage curve of Da accumulation calculated from the k_1 and k_2, where, on the curve of Da $(p\text{-ClPhS})_2$, the tangential line at inflection point and the induction period are shown for the explanation.

These values, however, merely indicate the chemical kinetic properties of Da derivatives *in vitro*. *In*

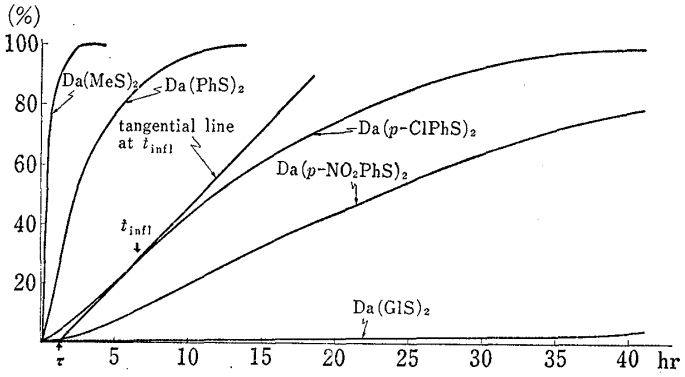


Fig. 8. The Calculated Curve of Da Liberation from Various MSDs at 37° and pH 7.44

Ph=phenyl

Table IV. Kinetic Parameters of Liberation of Free Da from Various Da MSDs at pH 7.44, 37°

	Da-$(HS)_2$	Da-$(MeS)_2$	Da-$(EtS)_2$	Da-$(GlS)_2$	Da-$(PhS)_2$	Da-$(p\text{-ClPhS})_2$	Da-$(p\text{-NO}_2\text{PhS})_2$
t_{infl} (hr)	unobtainable	0.333	0.311	84.0	0.869	6.56	11.35
$\left[\frac{d[Da]}{dt}\right]_{infl}$ (%/hr)	unobtainable	109.2	108.5	0.222	25.4	5.18	2.81
τ (hr)	unobtainable	0.0878	0.0861	20.0	1.23	1.36	3.25

vivo, the hydrolysis rate of MSD has been proved to be slower compared to that *in vitro*[5] and additionally the excretions of water-soluble forms, Da$(MS)_2$ and Da(MS), are suspected to be fast. In fact preliminary observation on the elimination from rabbit blood supports this suspicion, which will be reported later in the pharmacokinetic studies on these derivatives. Considering these results, it may be said that only a very minute amount of Da is utilized from the preparation of Da $(HS)_2$ or Da $(GlS)_2$. From the results on Da$(MeS)_2$, Da $(EtS)_2$, and Da $(PhS)_2$, these derivatives may be evaluated in the efficiency of Da liberation, and the faster hydrolysis may promise reduction of the administration dose. Da$(p\text{-ClPhS})_2$ and Da $(p\text{-NO}_2\text{PhS})_2$ were studied mainly from the interest on chemical kinetics, disregarding the physiological effect of the substituted groups.