formation. The control animals in this study showed no gross or microscopic pathology.

SUMMARY

A group of phthalic acid esters were studied for both acute and subacute toxicity in animals. The acute toxicity experiments included the evaluation of LD_{50}, hexobarbital sleeping time effects, rabbit intradermal tests, acute intravenous toxicity studies, and tissue culture effects. Subacute toxicity dealt with effect on body weight gain, organ–body weight ratios, effect on tissue of various organs, and effect on the hematopoietic system. Results of the study indicated that the group of phthalate esters reported in this paper had a low degree of toxicity when administered parenterally and that their degree of toxicity was parallel to their water solubility (greater solubility, greater activity) and to their molecular weight (lower molecular weight, greater activity). This low order of toxicity to parenteral administration appears to indicate that their use in applications implicated in this study are probably warranted and safe.

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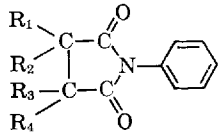
Hydrolytic Behavior of Some Alkyl-Substituted Succinanils

By A. K. HERD, III*, LENNART EBERSON†, and TAKERU HIGUCHI

The mechanisms and the relative rates of alkaline hydrolysis of succinanil and methyl-substituted succinanils have been investigated partly to help to elucidate the mechanism of carboxyl facilitated formation and hydrolysis of amides observed in these laboratories and also because a number of important hypnotics possess similar structures. As may be expected, the observed rates for the several imides roughly parallel those found for the corresponding anhydrides, succinanil being more reactive than the methyl-substituted compounds. The relative rates were in the order: unsubstituted > monomethyl > mesodimethyl > 2.2 dimethyl > racemic dimethyl > trimethyl > tetramethyl; the first member of the series reacting 83 times faster than the last. The tetramethyl anil was found to be sufficiently stable to co-exist as the major species in equilibrium with its cleaved product at pH of 8.

ALTHOUGH a number of cyclic imides, including glutethimide, methsuximide, phensuximide, etc., are widely used as drugs, relatively little has appeared in the literature concerning the rate of hydrolysis of such imides. Results of studies on the effect of structure on some aspects of this hydrolytic reaction are presented at this time. In particular, the investigation has been concerned with the influence of alkyl substitution on the rate of hydrolysis of *N*-phenyl succinimides (succinanils) (I). These reactions are of interest



I

R_1, R_2, R_3, R_4, = H or alkyl

not only because of the close relationship of these compounds to pharmaceuticals but also because

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they shed light on possible mechanisms of biochemical reactions.

It has been long recognized that methyl substitution generally increases ring stability. Thorp and Ingold and their students spent 15 years collecting evidence to substantiate this effect (1). Whether methyl substitution leads to greater thermodynamic stability for cyclic compounds than for their open-chain counterparts has not, however, been established with certainty. There is, nevertheless, evidence that methyl substitution aids in stabilization of the cyclic structure by accelerating the rate of ring closure (2). It had not been definitely ascertained, however, that methyl groups also stabilize the cyclic forms by inhibiting the rate of ring opening. Studies comparing anhydrides of dicarboxylic acids with their methyl-substituted analogs indicate that this may be the case (3). Whether this phenomenon is due to a steric hindrance to the attacking nucleophile or a steric hindrance to the linear separation of the 2 carboxylate groups is not yet clear.

Imide hydrolysis in general has not been the subject of great interest in recent literature, and hydrolysis of cyclic imides has received even less attention. Edward and Terry (4) have investigated the hydrolysis of succinimide in basic solutions and have shown that the rate of hydrolysis is first order in hydroxyl ion and first order in undissociated succinimide. Zerner and Bender (5) have shown that the hydroxyl-ion concentration is between pH 3.5 and 6.5.

EXPERIMENTAL

Materials.—The succinanils used in this investigation were prepared according to the method described by Fieser (6) for succinanil and were recrystallized from distilled water. Melting points of the products were compared with values given in the literature. Since the preparative method was straightforward, a good correspondence with melting points given in the literature was assumed as sufficient evidence for the existence of the compounds.

The dioxane used in this investigation was purified and made anhydrous by the treatment of technical grade dioxane as described by Vogel (7). Barium hydroxide solutions were prepared from carbon dioxide-free distilled water and sealed under nitrogen in glass ampuls. These samples were standardized against standard hydrochloric acid solutions. Corrections for appropriate ionic strength and temperature as affecting the partial dissociation of $Ba(OH)^+$ were estimated from the data of Gimblett and Monk (8).

The nitrogen which was used in the kinetic runs on the pH stat was passed over an Ascarite plug and bubbled through aqueous potassium hydroxide solution to remove carbon dioxide and prehumidified in a saturator at the reaction temperature. All the water used in these kinetic studies was freshly boiled and carbon dioxide free.

All other chemicals used in the preparation of buffers, kinetic solutions, and analytical reagents were analytical or reagent grade.

Kinetic Procedures for Hydrolysis of Succinanil and Methyl Succinanil.—The hydrolytic rate constants for these 2 compounds as a function of pH and temperature were determined in phosphate buffers of ionic strength 1.0 in a thermostated oil bath. Since it was found that the phosphate buffer catalyzed the hydrolysis of these compounds, each experiment involved determination of the hydrolysis rate at 5 buffer concentrations at the same pH and extrapolation to zero buffer concentration. The reaction solutions consisted of 99 ml. of the appropriate concentration of phosphate buffer and 1 ml. of a 1.2% solution of the anil in dioxane. The dioxane solution was added after the buffer solution had reached the required temperature in the oil bath. Five-milliliter samples were withdrawn periodically and quenched by diluting to 25 ml. with 0.25 *M* phosphate buffer at pH 6.0. These samples were analyzed spectrophotometrically at 240 mμ which is $\lambda_{max.}$ for the anilic acids. The appearance of the anilic acid was followed in this manner, and plots of $\log (A_\infty - A)$ *versus* time yielded straight lines from which k_{obs} could be calculated. Figure 1 shows plots of k_{obs} *versus* buffer concentration for succinanil at several pH values.

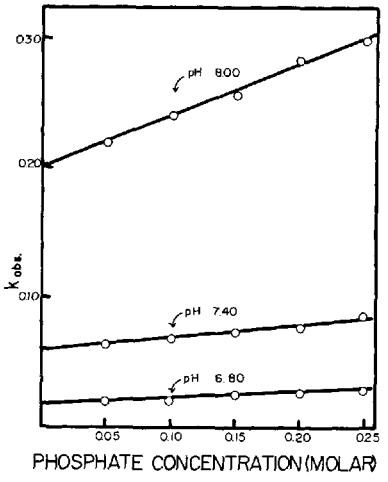


Fig. 1.—Plots of k_{obs} as a function of buffer concentration for hydrolysis of succinanil at 40°.

Kinetic Procedures for Hydrolysis of Methyl; meso-2,3-Dimethyl; 2,2-Dimethyl; Racemic 2,3-Dimethyl; and Trimethyl Succinanils.—The hydrolytic rate constants for this group of compounds were determined by use of a Radiometer TTT 1 automatic titrator with a SBR2 type Titrigraph and a TTA 3 titration assembly. Twenty-five milliliters of carbon dioxide-free water containing 1.45 Gm. of potassium chloride was placed in a water-jacketed beaker attached to a constant-temperature water bath. When the temperature had equilibrated, 1 ml. of a 1.2% solution of the anil in dioxane was added by means of a syringe and needle. The pH of the solution was maintained by the automatic addition of 0.025 *N* sodium hydroxide solution. Since the ml. of base added is directly proportional to the

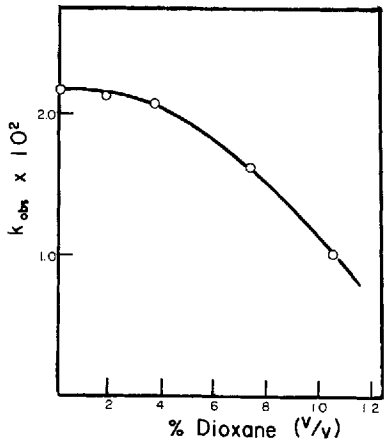


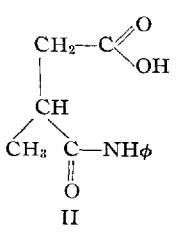
Fig. 2.—Plot showing dependence of k_{obs} for hydrolysis of methyl succinanil on dioxane concentration at 40° and pH 8.85.

amount of anilic acid being produced, plots of log (ml.$_\infty$ − ml.) *versus* time yielded straight lines from which k_{obs} could be calculated. Spectrophotometric analysis indicated that the reaction had essentially gone to completion.

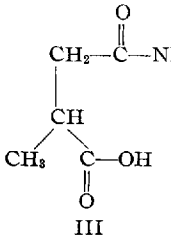
Since dioxane was used in these kinetic runs, the influence of dioxane concentration on k_{obs} was evaluated. Figure 2 shows values of k_{obs} for methyl succinanil as a function of dioxane concentration. At dioxane concentrations used in these experiments (3.84%), k_{obs} differed only slightly from k_{obs} at zero dioxane concentration.

Kinetic Procedure for Hydrolysis of Tetramethyl Succinanil.—Because of its slow rate of hydrolysis at lower pH values and because of the limitations of the pH stat method at higher pH values, the hydrolytic rate constants for tetramethyl succinanil were obtained in barium hydroxide solutions and were carried out directly in the sample cell of a thermostated Cary model 11 spectrophotometer. Twelve microliters of a 1.5% solution of the anil in dioxane was used for each run, along with 3 ml. of the barium hydroxide solution. The formation of tetramethyl succinanilic acid was followed by the change in absorption at 240 mμ. Plots of log (A_∞ − A) *versus* time yielded straight lines from which k_{obs} was calculated. Spectrophotometric analysis at the end of each run indicated that the anil had essentially hydrolyzed completely to the anilic acid.

Identification of Reaction Products.—All reaction products were identified spectrophotometrically. However, it must be mentioned at this time that in the hydrolytic cleavage of certain of the anils studied in this series, 2 different products are possible. For example, hydrolytic cleavage of methyl succinanil can yield the 2 isomers, II and III.



II



III

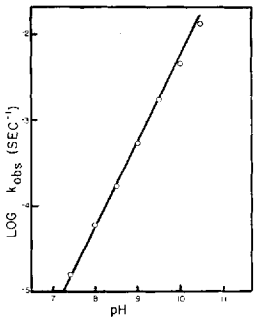


Fig. 3.—pH rate profile for hydrolysis of methyl succinanil at 40°. The solid line has been drawn with a slope of 1.00.

Probably a mixture of II and III is actually produced. Although one may expect purely on steric and electronic considerations that compound II would be the more prominent species, in this investigation no attempt was made to isolate and identify the products. The same was also true for 2,2-dimethyl and trimethyl succinanil.

RESULTS AND OBSERVATIONS

The second-order rate constants for a series of methyl-substituted succinanils were determined at temperatures ranging from 25 to 55°, and the kinetic parameters E_a and $\Delta S^\ddagger$ were calculated. From past work it might be expected that the hydrolysis would be independent of pH at low values (5), but at the pH values employed in this investigation, the reaction has been shown to be first order with respect to hydroxyl-ion concentration. It would appear, therefore, that the rate constants which are to be compared all stem from an identical mechanism. Figure 3 shows a pH-rate profile for the hydrolysis of methyl succinanil at 40°.

Table I lists all the anils studied in this investigation and their second-order rate constants calculated at 25°. The apparent E_a and $\Delta S^\ddagger$ values are shown also. The $\Delta S^\ddagger$ values were all calculated at 25°. The E_a values listed in this table for the first 6 compounds are 12.6 Kcal. less than those values calculated from the slopes of Arrhenius-type plots for runs made at constant pH. This accounts for the change in K_w with temperature.

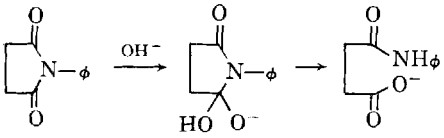
DISCUSSION

The observed markedly decreased rate of hydrolysis of the succinanils induced by methyl substitution is not altogether unexpected. Although the nature of the activated states involved in the cleavage of these imides differs significantly from those responsible for formation of hydrolysis of the amic acids, there are some similarities. Considering the

TABLE I.—RATE CONSTANTS FOR OH$^-$-CATALYZED HYDROLYSIS OF ANILS AT 25°

Compd.	k (1/m/sec.)	E_a, Kcal./mole	$\Delta S^\ddagger$, (E.U.)
Succinanil	5.45	12.3	−15.9
Methyl-	5.20	12.8	−15.9
meso-2,3-Dimethyl-	4.98	10.0	−19.5
2,2-Dimethyl-	3.57	10.6	−22.2
Racemic 2,3-dimethyl-	2.92	11.2	−20.7
Trimethyl-	0.815	11.7	−22.0
Tetramethyl-	0.0669	9.4	−34.3

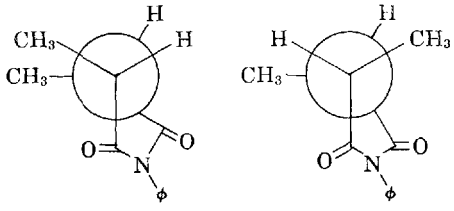
second-order character of the reaction, one can write Scheme I.



Scheme I

From this postulated mechanism, it is obvious that there are 3 general phenomena which could explain the decrease in hydrolytic rate with increased methyl substitution: (*a*) polar contribution of the methyl groups which would tend to reduce the $\delta+$ charge on the carbonyl carbon, thus reducing the rate of hydroxyl attack; (*b*) steric inhibition by the substituted methyl groups to the attacking nucleophile; and (*c*) steric hindrance to ring opening due to the presence of the methyl groups.

This last phenomenon has been pointed out by Bordwell and co-workers (9) in their studies on sultones. These workers show large decreases in rates of hydrolysis with increasing methyl substitution; they attribute this effect largely to steric hindrance of ring opening. They argue that in sultone hydrolysis, the ring opening must occur by a rotation about the bonds holding the severed groups, and that methyl substitution restricts this rotation. This line of reasoning may be applied in the present investigation to explain the difference in hydrolytic rates for the *meso* and racemic isomers of 2,3-dimethyl succinanil. Examination of Newman projections of these compounds indicate that in the *meso* isomer (IV) the methyl groups are already in an



meso IV

Racemic V

eclipsed position, and a rotation on cleavage would reduce this methyl–methyl interaction. On the other hand, a rotation in the racemic isomer (V) would increase methyl–methyl interactions. Since the polar effects and steric inhibition to nucleophilic attack in these two isomers would be expected to be similar, the steric inhibition to ring opening seems to be a reasonable approach for explaining the difference in hydrolytic rates of these 2 compounds (Table I).

Bruice and Pandit (3) have investigated the solvolysis of certain cyclic anhydrides, as well as the solvolysis of mono esters of these anhydrides and concluded that although a restriction of rotation of reacting groups away from each other markedly increases the rate of anhydride formation, it does not decrease the rate of anhydride solvolysis. They point out that although certain mono-esters of maleic acid form the anhydride about 50 times faster than the corresponding succinic monoesters, maleic anhydride solvolysis proceeds about 8 times faster than that of succinic anhydride. Their findings do not actually refute the suggestions of Bordwell *et al.*, since maleic anhydride hydrolysis would not involve a rotation but would probably relieve a strain imposed by the cyclic structure.

From the previous discussion and from the data presented in this investigation, it becomes apparent that no definite conclusions can be drawn concerning the differences in the hydrolytic rates of methyl-substituted succinanils at this time. This, of course, is due to the fact that the 3 possible phenomena which may be responsible for the change in rates cannot be separated. It can be suggested, however, that polar effects and steric inhibition to nucleophilic attack are mostly responsible for the differences in rates among the lower methyl-substituted compounds in this series and that a "steric hindrance to ring opening" effect becomes more pronounced as methyl substitution is increased. This is based on the fact that the relative rates change only slightly in the lower methyl-substituted members, while there is a greater rate change in the tri- and tetramethyl-substituted members of this succinanil series.

SUMMARY

Although hydrolytic cleavage of succinanils is not strictly related to anhydride formation occurring during the formation and hydrolysis of the succinanilic acids in dilute aqueous solutions, certain similarities are thought to exist. The hydrolytic rate constants were determined in aqueous buffered solutions for succinanil and methyl succinanil; in barium hydroxide solutions for tetramethyl succinanil; and by use of a pH stat for methyl, *meso*-2,3-dimethyl, 2,2-dimethyl, racemic 2,3-dimethyl, and trimethyl succinanil.

The fact that increased methyl substitution decreases the rates of hydrolysis in this series was not unexpected in light of previously compiled data. The interpretation of these results appears to be rather complicated since there were probably several factors, such as polar effects, steric inhibition to nucleophilic attack, and steric hindrance to ring opening, which would tend to decrease the hydrolytic rate. Although it was not possible to separate these effects, it is suggested that the last effect became more prominent as methyl substitution was increased.

The findings of this investigation seem to indicate that not only does methyl substitution markedly enhance ring formation, but it is also responsible for increased ring stability.

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