[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF THE UNIVERSITY OF CALIFORNIA]

BRANCHED-CHAIN FATTY ACIDS. XI. LOCATION OF BRANCHING METHYL GROUPS NEAR CARBOXYL BY RATE STUDIES OF AMIDE HYDROLYSIS

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The location of branching groups in aliphatic hydrocarbon chains has remained a rather difficult problem, although there have recently been developed methods which are quite effective in some instances. Infra-red spectroscopy (1), X-ray diffraction (2), and monolayer studies (3) have been applied effectively, and phase diagrams between branched and straight-chain acids have been used to great advantage (4) in locating branching groups near the tail end of an acid chain. In some favorable examples, oxidative cleavage of the chain has been of value. The 10-methyl group (5) in tuberculostearic acid was first located in this manner, but a similar oxidation (6) of phthioic acid supplied only limited information. Any methods which can be applied to aliphatic acids are of considerable general utility, since acids are often obtained as degradation products in instances where the carboxyl group was not originally present in the molecule under investigation.

Since aliphatic amides containing an α-methyl group and a second large α-alkyl group have, thus far, resisted all efforts (7) to hydrolyze them to the corresponding acids, it would seem entirely possible that a single methyl substituent would have an observable effect on the rate of hydrolysis of an amide. The amide should be an ideal derivative for such a study, for it is nearly always crystalline, hence suitable for careful purification, and may usually be obtained in nearly quantitative yield (8) from the acid. Furthermore, the acid is quantitatively recovered if hydrolysis is carried to completion, an important consideration for study of natural products. Rate of hydrolysis may be easily followed by determination of ammonia evolved.

A survey of the literature has failed to reveal anything of interest concerning the hydrolysis of branched-chain amides; however, there have been reported pertinent investigations of ester hydrolysis in the presence of alkali. Of the several reports, those of Levenson and Smith (9) and of Evans, Gordon, and Watson (10) appear most authoritative, since the results in these two investigations are not only self-consistent but reasonably consistent with each other. The work of Bryant and Smith (11) is also of interest, but is handicapped by their use of commercial esters without purification. The work of these investigators shows that (a) the rate of hydrolysis of ethyl esters of normal acids remains constant in the molecular weight range from butyric to lauric acid, hence, presumably, for all higher molecular weights; (b) a substituent methyl group in the *alpha* or *beta* position decreases the rate to about one-fourth the value for a straight-chain ester; (c) a methyl substituent in the *gamma* position does not affect the rate. Since the branched-chain esters investigated were of acids con-

taining six or less carbons, the relative effect of an *alpha* or *beta* methyl was obscured by the large effect of varying the size of the second *alpha* substituent. For example, the ethyl ester of dimethylacetic acid hydrolyzes at about fifty times the rate of the ethyl ester of diethylacetic acid, although ethyl caproate hydrolyzes more rapidly than ethyl dimethylacetate. Ethyl β-methylbutyrate hydrolyzed slightly more rapidly than did ethyl α-methylbutyrate.

The results summarized above indicate that rate of amide hydrolysis should be quite effective in detecting the presence of a methyl substituent in the *alpha* or *beta* positions of higher-molecular-weight acids. The work described in the present paper, using the pure branched-chain amides whose synthesis has been reported (8), shows that these expectations may be realized. In fact, it is also possible to determine whether the substituent is *alpha* or *beta*, for the β-methyl amide hydrolyzes *more slowly* than does the α-methyl amide, in spite of the fact that the γ-methyl amide hydrolyzes at the same rate as a normal amide. This somewhat surprising result is entirely inconsistent with the suggestion of Evans, Gordon, and Watson that the effect of alkyl substituents in retarding rate of hydrolysis should be ascribed to the electron-repelling tendency of alkyl, which makes the carbonyl carbon less positive. A similar explanation has been offered by Gordon, Miller, and Day (12) for the effect of an alkyl substituent in decreasing the rate of ammonolysis of esters. It seems difficult to explain how any inductive effect of the alkyl substituent could be greater in the *beta* position than in the *alpha* position, for it is well established that inductive effects in aliphatic molecules fall off very rapidly with distance. It is still more difficult to explain how the effect could abruptly drop to zero in the *gamma* position.

These facts may be correlated if an alternative explanation is adopted, namely, that the retarding effect of an alkyl substituent on hydrolysis is largely or entirely steric in nature. If scale (Fisher-Hirschfelder) molecular models are constructed for the α-, β-, and γ-methyl amides it is apparent that the β-methyl offers more interference with the free rotation of the amide group than does the α-methyl, whereas the γ-methyl presents no interference to this rotation. These facts are reflected in the rates of hydrolysis of these amides; however, it should be emphasized that this interpretation is a qualitative one. If there is assumed a random distribution of the groups in all possible positions resulting from rotation about the valences, it is not immediately apparent why the hindering effect of the α- and β-methyl groups is as large as that observed. If, however, the attack of hydroxyl on the carbonyl carbon should be of the "face-centered" type, similar to that involved in substitutions leading to Walden inversion, then the amount of hindrance observed seems not unreasonable. Evidence for operation of this type of hindrance in substitution reactions has been presented and discussed by Bartlett and Rosen (13).

As regards the application of these observations to structure determination, it has also been found that an α,β-dimethyl amide hydrolyzes much more slowly than an amide with a methyl in either of these positions. It has been confirmed that the rates at this molecular weight level are independent of molecular weight. Straight-chain amides with 18, 19, or 25 carbons gave the same rates of hydrol-

ysis, as did α-methyl amides with 19 or 23 carbons, and β-methyl amides with 19 or 25 carbons. The apparent rate constants, illustrating these facts, are found in Table II. The variation of rate of hydrolysis with structure is graphically illustrated in Figure 2.

In a previous communication (14) it has been pointed out that if phthioic acid (6), the biologically active branched-chain acid from tubercle bacillus, is a long chain with several branching methyl groups, then two of these groups are probably in the *alpha* and *beta* positions. By use of the amide hydrolysis method, it should be possible to settle this point quickly. The rate data should fit one of the curves in Figure 2, unless the material is either non-homogeneous, or there is a larger group or ring at the *alpha* or *beta* (or possibly *gamma*) position. We are, at present, attempting the isolation of phthioic acid for such experiments.

So far as concerns the phthioic acid problem, there is no interest in acids with two methyl groups on the *alpha* or *beta* carbon, for the high optical rotation of phthioic acid cannot be reconciled with such a structure. It is planned, however, to extend the generality of the method by studying the hydrolysis of amides bearing two methyls on one carbon, and those having larger alkyl substituents.

METHOD AND CALCULATIONS

Materials. The preparation of all the amides used has been described (8), except for stearamide, which was prepared from stearic acid purified by distillation of the ester and recrystallization of the acid.

Discussion of method. A relatively concentrated solution was found most convenient and sufficiently accurate for our purposes. Hydrolysis with 0.25 *N* potassium hydroxide in boiling ethanol was found to be inconveniently slow; so the rate was increased to a convenient value by use of 0.5 *N* potassium hydroxide in boiling 1-propanol. This permitted a precision of about 5% in determination of the rate constant, except for the faster rates of the normal and 4-methyl amides, where the precision is about 10%. These limits of precision are adequate for present distinctions. If finer distinctions become necessary (for example, in investigating possible differences between normal and 4-methyl amides) the accuracy may be increased by use of more dilute solutions and the lower temperature of boiling ethanol. A principal reason for the lower accuracy for the faster rates is that the time elapsing during distillation (*cf.* Procedure) is a relatively large percentage of the total time, and the boiling point is raised slightly during this period, by concentration of the solution.

It was found that the rate constant was increased appreciably when the sample size was reduced by one-half, and it was suggested by Professor R. E. Connick, of this Laboratory, that this might be caused by a decrease in the amount of paraffin-like material (the amide or salt of the acid) in solution. At the concentrations used by us (0.035–0.07 molar), this might change the nature of the solvent sufficiently to affect the activity of the hydroxide ion. This appears to be the case, for when 0.035 molar concentration of amide and 0.035 molar concentration of *n*-hexadecane were used, the rate was the same as when 0.07 molar concentration of amide was used. These facts are illustrated by curves I and II, Figure 2, and by the data on 2-methyloctadecanamide, Table II.

For a set of runs made consecutively with the same normality of alkali and the same size of sample, the type of structure may be most easily determined by a simple plot of time against per cent of amide hydrolyzed, as in Figure 2. Also, this is probably the most accurate method of comparison; however, it is subject to some difficulties, especially since the normality of alcoholic potassium hydroxide changes rapidly on standing. For this reason, our most complete comparisons are on the basis of apparent rate constants (Table II), ex-

pressed in liters moles^{-1} hours^{-1}. In Figure 2, the excessive slope of the curves (especially V) between zero and the first point indicates an irregularity at the beginning of the hydrolysis; however, this is no disadvantage if the zero time point is not used in the plots (as in Figure 3) for determination of rate constants. The remaining points gave a satisfactory straight line plot, in all cases.

A few runs were carried to completion, and the total ammonia titrated checked the theoretical amount with an accuracy of 2% or better. Several runs were made on 0.1-g. samples

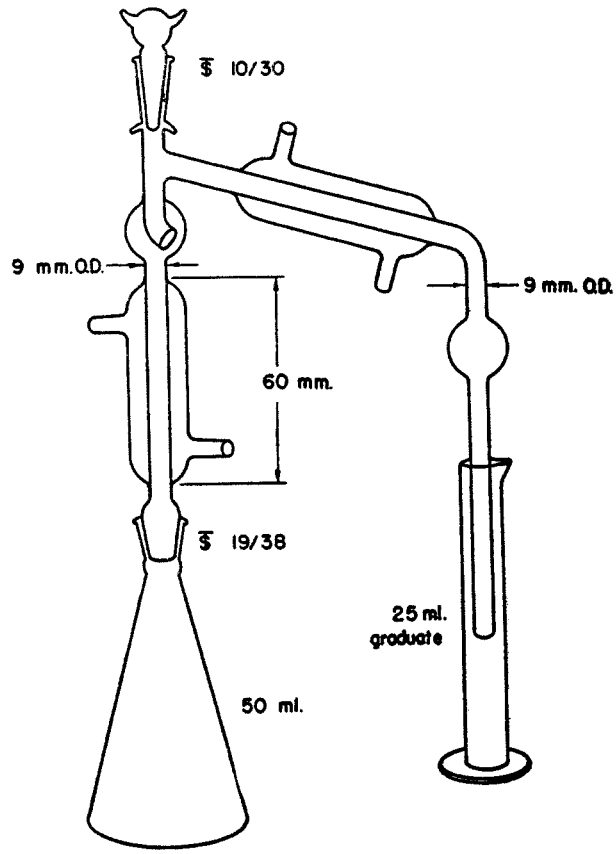

FIG. 1. APPARATUS FOR AMIDE HYDROLYSIS

with sufficient accuracy to distinguish the type of amide, but the plot for determination of rate constant was rather inaccurate.

Procedure. The simple apparatus shown in Figure 1 was used. For a run, the sample (0.25–0.5 g.) of amide was weighed accurately into the flask, and 25 ml. of standardized, approximately 0.5 *N* potassium hydroxide in 1-propanol was pipetted into the flask. In the graduated cylinder was placed 10 ml. of 2% aqueous boric acid solution. The mixture in the flask was then heated under reflux on a hot plate, with water circulating through the upright condenser. When a determination was to be made, water was drained from the upright condenser and 10 ml. of alcohol was distilled rapidly (6–10 mins.) in order to carry over all ammonia. Time was taken at the point when distillation was stopped by running water through the upright condenser, and 10 ml. of 1-propanol was immediately added through the ground joint at the top. The graduate was lowered for the last 2 ml. of distillate and the outside of the delivery tube was rinsed with carbon dioxide-free water. A fresh sample of boric acid was then arranged to receive ammonia.

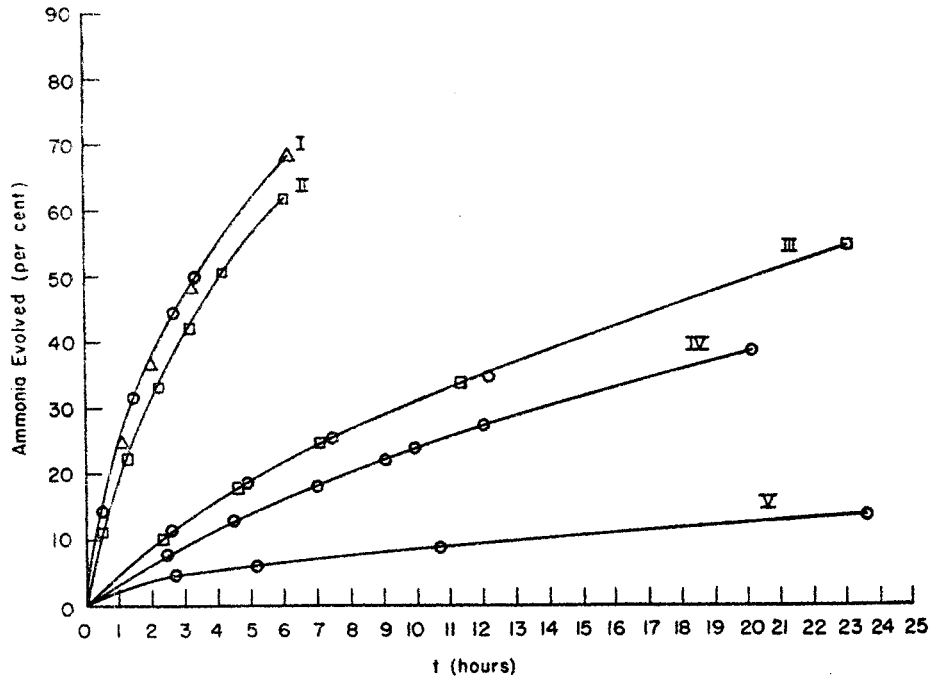

FIG 2. RATE OF HYDROLYSIS OF AMIDES IN APPROXIMATELY 0.5 *N* KOH IN 1-PROPANOL. Curve I: △—△, 0.035 molar stearamide; ⊙—⊙, 0.034 molar 4-methyloctadecanamide. Curve II: ⊡—⊡, 0.071 molar stearamide. Curve III: ⊡—⊡, 0.034 molar 2-methyldocosanamide; ⊙—⊙, 0.034 molar 2-methyloctadecanamide. Curve IV: 0.034 molar 3-methyltetracosanamide. Curve V: 0.031 molar 2,3-dimethyloctadecanamide.

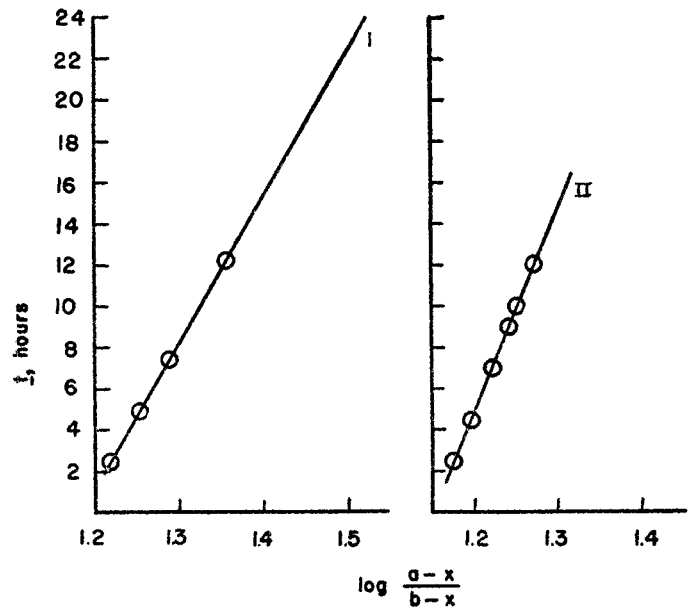

FIG. 3. DETERMINATION OF SLOPE FOR RATE CONSTANTS. Curve I: 2-Methyloctadecanamide. Curve II: 3-Methyltetracosanamide.

The distillate was rinsed into a 125-ml. Erlenmeyer flask with 25 ml. of carbon dioxide-free water, and the ammonia was titrated directly with 0.02 N hydrochloric acid, using the excellent Tetrabromophenol Blue and Methyl Red indicator of Stover and Sandin (15). A blank titration was made and subtracted, according to the method of these authors.

The time interval at which titrations were made was adjusted to the rate of the hydrolysis being studied. For normal or 4-methyl amides, titrations were made at intervals o

TABLE I

CALCULATIONS FOR RATE CONSTANTS, 2-METHYLOCTADECANAMIDE

t (HRS.)	x	$a - x$	$b - x$	$\log \frac{a-x}{b-x}$
2.58	0.0038	0.494	0.0298	1.219
4.90	.0063	.492	.0273	1.256
7.48	.0086	.489	.0250	1.291
12.22	.0123	.486	.0213	1.358

$a = 0.4984$

$$\text{slope} = \frac{24.0 - 4.6}{1.525 - 1.250} = 70.6$$

$b = 0.0336$

$$K = \frac{2.303}{(0.4648)\ (70.6)} = 0.070.$$

3-METHYLTETRACOSANAMIDE

t (HRS.)	x	$a - x$	$b - x$	$\log \frac{a-x}{b-x}$
2.50	0.0027	0.4728	0.0316	1.175
4.50	.0045	.4710	.0298	1.199
6.98	.0062	.4693	.0281	1.223
9.05	.0077	.4678	.0266	1.245
9.98	.0082	.4673	.0261	1.253
12.05	.0094	.4661	.0249	1.272

$a = 0.4755$

$$\text{slope} = \frac{15.0 - 5.0}{1.303 - 1.202} = 99.0$$

$b = 0.0343$

$$K = \frac{2.303}{(0.4412)\ (99.0)} = 0.0527.$$

30–60 minutes; for the slower hydrolyses, samples were taken at intervals of 1–4 hours. Four to six titrations were commonly made. The amount of boric acid used will absorb about 9 mg. of nitrogen quantitatively (15).

All 1-propanol used was a distilled commercial grade, b.p. 94–99°. The potassium hydroxide solution was standardized each day that it was used. The normality changed about 0.0005 units per day.

Calculation of rate constants. The second order apparent rate constant, k, for the temperature of the boiling solution was calculated from the equation:

$$t = \frac{2.303}{k(a - b)}\left(\log \frac{b}{a} + \log \frac{a - x}{b - x}\right)$$

where a is the initial molarity of alkali, b is the initial molarity of amide, and x is the moles reacted in time, t. By plotting $\log \frac{a-x}{b-x}$ against t in hours, the equation was reduced to the form:

$$k = \frac{2.303}{a-b}\left(\frac{1}{\text{slope}}\right)$$

The plot gave a satisfactory straight line for points taken up to the half time of the reaction. The points naturally became less reliable, as x approached b. It was felt that our accuracy is not such, nor our times sufficiently long, to justify a "glass correction," as used by Levenson and Smith (9).

TABLE II

APPARENT RATE CONSTANTS FOR SAPONIFICATION OF AMIDES

AMIDE	0.067 MOLAR[a] AMIDE	0.034 MOLAR[a] AMIDE
Stearic	0.34	0.38
Nonadecanoic		.35
Pentacosanoic		.34
4-Methyloctadecanoic	.39	.40
2-Methyloctadecanoic	.058	.070
	.054	
		.054[b]
2-Methyldocosanoic	.059	.074
3-Methyloctadecanoic	.045	
3-Methyltetracosanoic		.053
2,3-Dimethyloctadecanoic		.013

[a] These figures represent the approximate molarity of amide, and in all runs the potassium hydroxide in 1-propanol was approximately 0.5 molar. Exact molarities were used for the calculations. The temperature is that of the boiling solution, about 99°.

[b] In this run, 0.034 molar concentration of n-hexadecane was added to the saponification mixture. Similar effects were observed in several runs with stearamide, but the data are less convincing on account of the lower accuracy for the faster hydrolyses.

Sample plots are shown in Figure 3, and in Table I are the calculated values from which the plots for 2-methyloctadecanamide and 3-methyltetracosanamide were made. Rate constants, k, are in liters moles^{-1} hours^{-1}. Data for the other amides were treated in a similar manner.

SUMMARY

It has been shown that the rate of amide hydrolysis is a convenient and rapid method for distinguishing between an α-methyl, a β-methyl, and an α,β-dimethyl amide. The rate for each of these is different, and all are slower than that for a normal or γ-methyl amide.

Since the β-methyl amide is hydrolyzed more slowly than the α-methyl amide, the retarding effect of the alkyl substituents is attributed to steric hindrance.

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