[Contribution from the Chemical Laboratory of the University of California]

BRANCHED - CHAIN FATTY ACIDS. XIV. LOCATION OF BRANCHING METHYL GROUPS BY STUDY OF THE MELTING POINTS OF BINARY MIXTURES OF BRANCHED AND NORMAL ACIDS OR AMIDES

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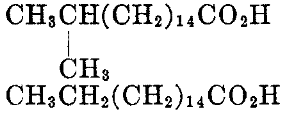
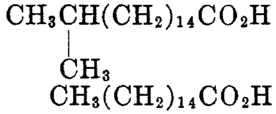
Branching methyl groups in the 2- or 3-positions of fatty acids may be definitely located by studying the rate of amide hydrolysis (1), and some information concerning the location of a branching methyl may be obtained from the melting points of the acid and its derivatives (2); however, there remains to be developed a general method for definitely locating a methyl group at any position along the chain. A promising method, which consumes relatively small quantities of materials, is the study of the melting or solidification points of binary mixtures of branched and normal acids, as introduced by A. W. Weitkamp (3).

In working with iso acids (isopropyl end-group) and anteiso acids (*sec*-butyl end-group), this investigator demonstrated the following two principles. (A) For mixtures of a branched-chain acid and a normal acid, if the total number of carbons in the normal acid is *greater than* the number of the carbon bearing the branching methyl, a melting-point diagram exhibiting *one* eutectic is obtained. (B) If the total number of carbons in the normal acid is *equal* to the number of the carbon bearing the branching methyl in the branched-chain acid, a melting-point diagram exhibiting *two* eutectics is obtained. This may be illustrated by considering the two pairs, A and B. Pair A exhibits one eutectic, pair B exhibits two.



$$\underset{\displaystyle CH_3}{CH_3CH}(CH_2)_{14}CO_2H$$
$$CH_3CH_2(CH_2)_{14}CO_2H$$

A



$$\underset{\displaystyle CH_3}{CH_3CH}(CH_2)_{14}CO_2H$$
$$CH_3(CH_2)_{14}CO_2H$$

B

A theoretical basis for this behavior was suggested and discussed.

Since this method seemed quite promising and was not being pursued further by Weitkamp, we have prepared the necessary acids (2) for an extension of these studies. It was of particular interest to learn whether the method could be applied to the location of branching methyl groups at positions other than the iso or anteiso positions. All compounds used in our investigations were *dl*-isomers except for *d*-10-methyloctadecanamide. Effects of stereoisomerism are discussed below.

We first repeated the determinations for a set of curves similar to those published by Weitkamp, to make certain that similar results could be obtained by

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us. Figure 1, showing our melting-point curves and the solidification-point curves of Weitkamp, demonstrates that the results are entirely consistent. The displacement of about 10% between the two sets of curves is probably caused by the known difference between the melting-point and solidification-point curves for fatty acid mixtures, although there may have been a small difference in the purity of the samples of 16-methylheptadecanoic acid. The shapes of the curves, as well as the number and location of eutectics, are very similar.

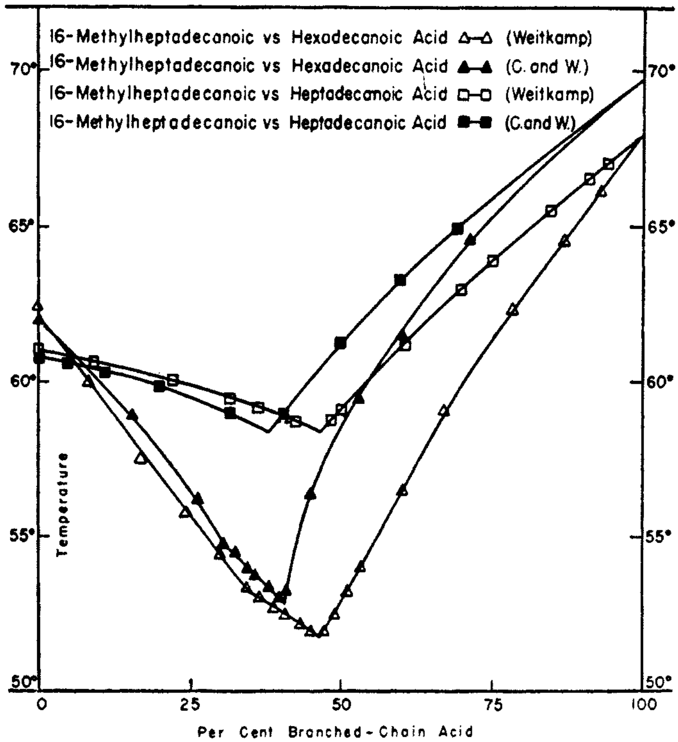

FIGURE 1

Weitkamp published one set of curves, using amides, and the results were similar to those obtained with the acids. As shown in Figure 2, we also obtained similar results with 16-methylheptadecanamide and hexadecanamide; in fact, the occurrence of two eutectics is considerably more pronounced with the amides. Also in Figure 2 is a curve showing that two eutectics are also obtained when the normal amide is shorter than the position of branching in the branched acid. The curve rises between the eutectics in this case, but the approximate compositions at the eutectics and the general trends in the two curves in this figure are similar. Since many of the acids studied in the present work have inconveniently low melting points, many of the curves have been determined on the amides.

As soon as our investigations were extended to acids with branching more remote from the end of the chain, it became apparent that the simple principles,

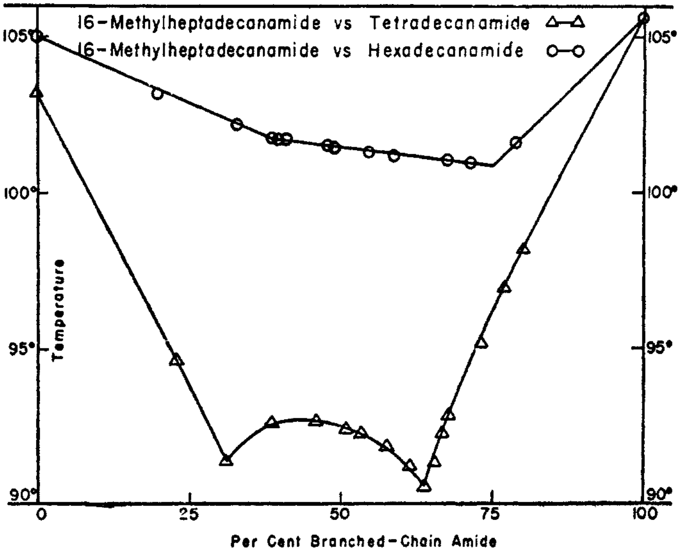

FIGURE 2

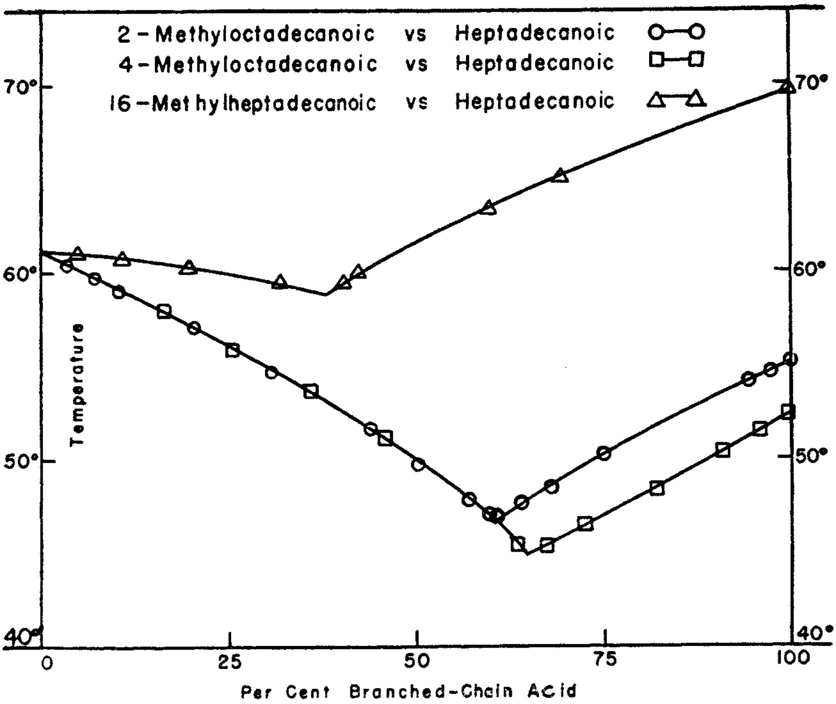

FIGURE 3

A and B, mentioned above, no longer applied. The curves were usually much more complicated, and the relationship between position of branching and chainlength of normal acid had lost its significance. It has been possible, however, to apply a different type of analysis and to arrive at useful generalizations.

Location of methyl groups by zones. By a study of the curves obtained with branched acids (or amides) and normal acids (or amides) of approximately the molecular weight of the branched acids, it is possible to locate the methyl group in one of three zones. A fourth zone consists of the iso and anteiso positions, where a definite location is possible, as previously mentioned.

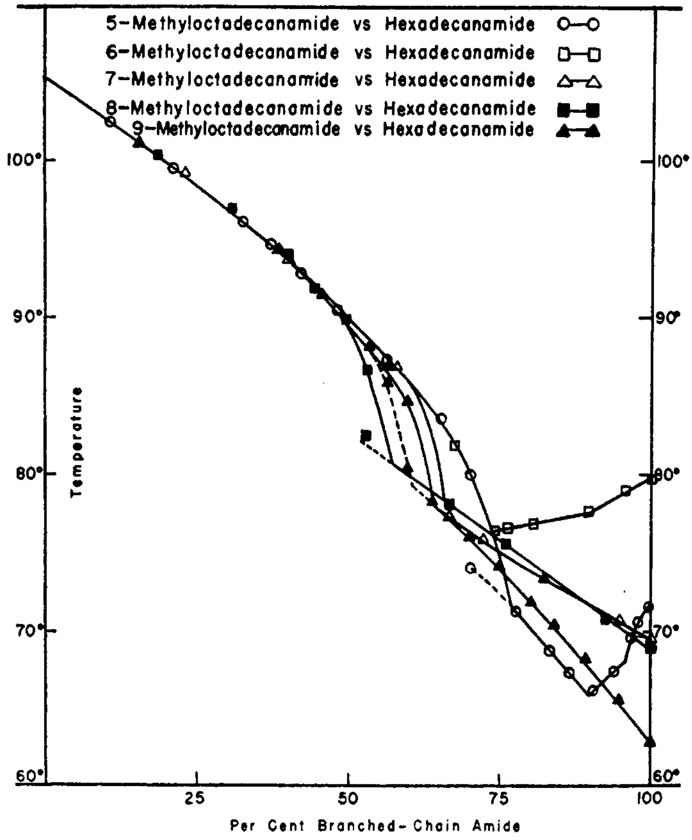

FIGURE 4

Positions 2-, 3-, and 4-. Inspection of Figure 3 shows that the curves with 2-methyl and 4-methyl acids are those characteristic of an ideal solution over the entire range. On the left of the eutectic, the species separating is heptadecanoic acid, and in the absence of solid solution this curve should be the same for any second component. This is the case for the two curves mentioned. Furthermore, the slope of the curve, in absence of solid solution, is a function of the heat of fusion[2] of the species crystallizing. Since 2-methyl- and 4-methyl-octadecanoic

[2] The data in this paper could be used to make plots for calculating heats of fusion for some of the acids studied, but this information is not regarded as of significance to the present study. The calculation would apply only in the few instances where an ideal solution over an appreciable range is indicated.

acids would be expected to have similar heats of fusion, the two curves on the right of the eutectics should be approximately parallel, and this is the case. These two curves are easily distinguished from the one-eutectic curve for 16-methylheptadecanoic acid, where there is a large deviation from ideality. It should be mentioned that distinction between substitution in positions 2-, 3-, and 4- is easily accomplished by investigation of the rate of amide hydrolysis (1).

Positions 5- and 6-. For all positions of methyl beyond the 4-position, there is a large deviation from ideality caused by both solid solution and molecular compound formation, the latter being responsible for multiple eutectics. In Figure 4, it will be noted that the curves for the 5-methyl and 6-methyl isomers go down from the origin at 100% branched-chain amide (right side). They differ markedly, however, from the curves described in the preceding section, in that they exhibit two and three eutectics, respectively. The eutectic at about 95% 5-methyloctadecanamide will be discussed below in the section on the effects of stereoisomerism.

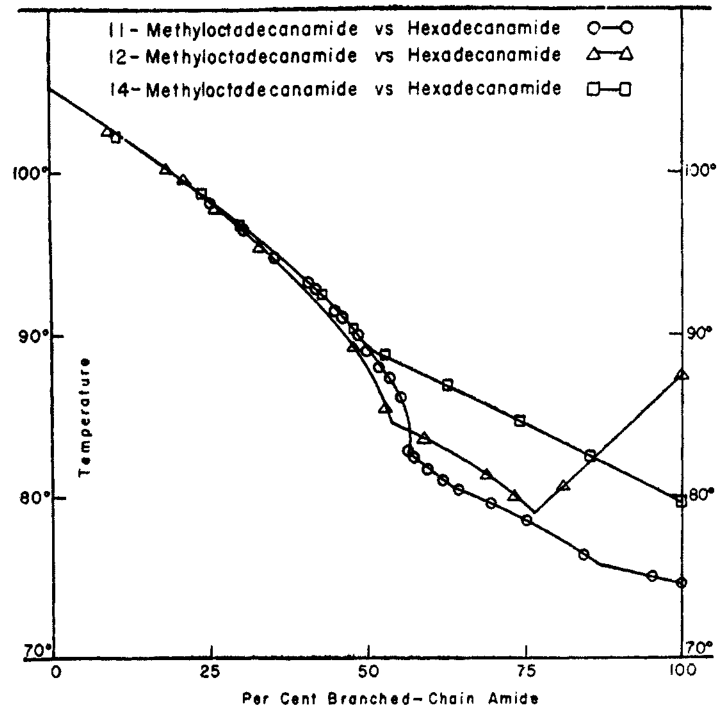

FIGURE 5

In three of the curves in Figure 4 are plotted *double melting points.* The melting point obtained depended on the history of the solution prior to melting, and this is discussed in the section on experimental procedure. The lower melting point is attributed to a metastable polymorph or molecular compound, and these points have been joined by dotted lines.

Positions more remote from carboxyl than 6-. For positions more remote from carboxyl than 6-, it will be noted in Figures 4, 5, and 10 (Figure 10 is discussed in detail below) that, with one exception, the curves all *rise* from the origin at 100% branched-chain amide (right side). This is not the case in any curve with a branch nearer the carboxyl than position 7-, so these two categories are easily distinguished. The failure of the 12-isomer to follow this behavior of all its neighbors may be due to the unusually high melting point of the amide of this acid. In any case, there is no difficulty in distinguishing the 12-isomer from the 5- and 6-isomers, for the 12-methyloctadecanoic acid melts considerably below the 5- and 6-isomers, and is polymorphic, while the amide of the 12-methyl isomer melts unusually high. In fact, its unique properties make the 12-methyl isomer especially easy to detect.

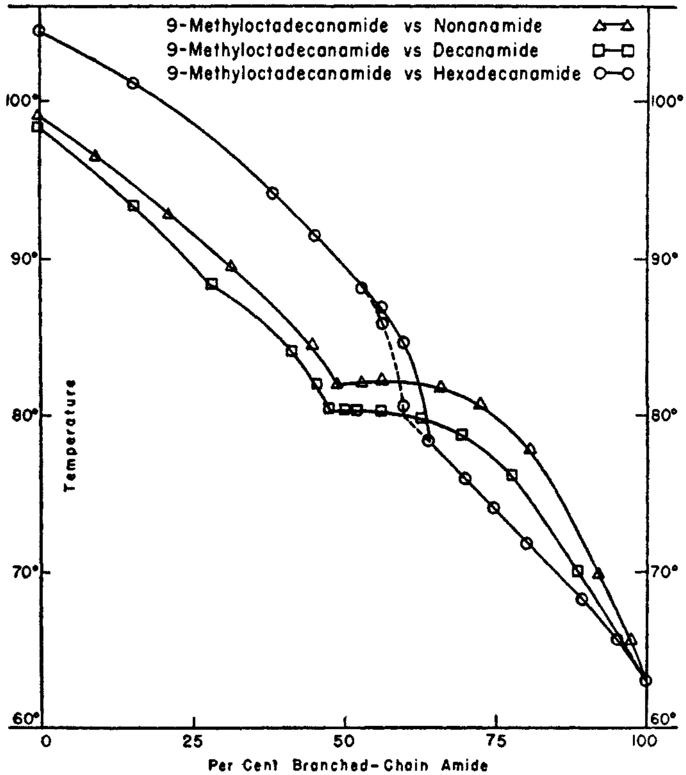

FIGURE 6

Exact location of methyl groups. The data discussed above makes possible the exact location of methyl groups in many positions, but the positions (except 12-) between 6- and anteiso remain lumped together in one category. A study of the curves in Figures 6–10 suggests a method for locating the position of a branching methyl in this central portion of the chain. The number of curves in each figure is such that they are crowded in the diagram, but this serves to point up the most interesting feature of these groups of curves. For each position of methyl, a curve of characteristic shape is obtained, regardless of which normal acid is used as the second component of the mixture. Thus, all curves in Figure 6 are of similar shape with a eutectic in the region between 45% and 65% branched-chain amide. All curves in Figure 7 show two eutectics, one in the region between 40% and 55%, the second at 90–95%. The curves in Figure 8 also show two eutectics, but the second is at 75–85%. The curve with tridecanamide, in Figure 8, is notable in that it is double from the first eutectic over the remainder of the curve. This is still another unique feature of the 12-methyl isomer. Finally, the

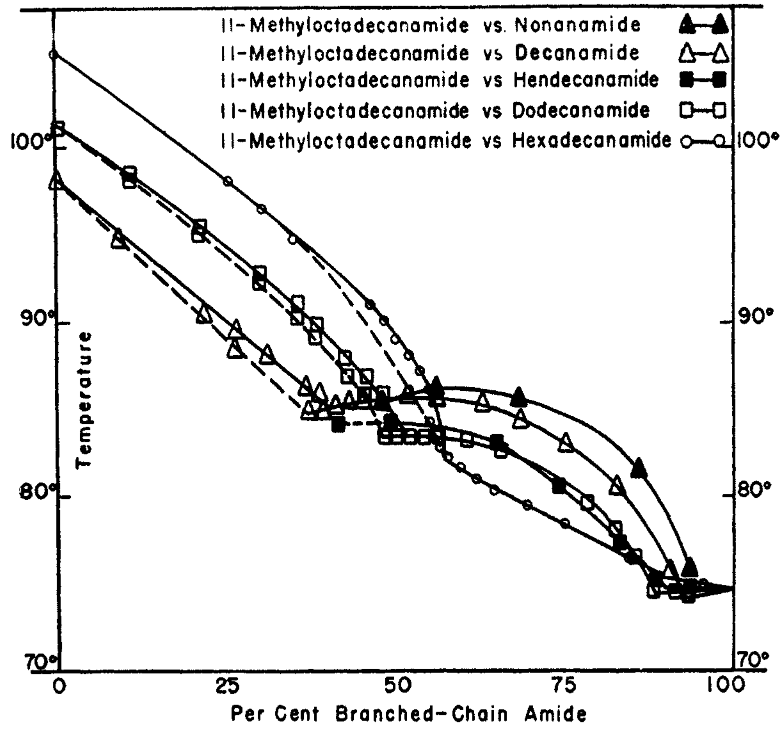

FIGURE 7

curves in Figure 9 also show two eutectics, one at 25–30%, the other at 40–55%. In Figure 9, there may be a second eutectic in the curve with hexadecanamide, at about 25%, but the inflection was so slight that its existence is doubtful, so it is not indicated on the graph. Two curves in Figure 7 and one curve in Figure 8 are left incomplete, to relieve the crowding, but the part shown is enough to demonstrate the similarity of their shape to the completed curves.

Thus, the position of a methyl in an unknown acid can be detected by constructing one curve with a normal acid of about ten carbons. By inspection, it can be determined which type of curve has been obtained, and the position of the methyl thus located. This procedure is similar to the application of ultra-violet

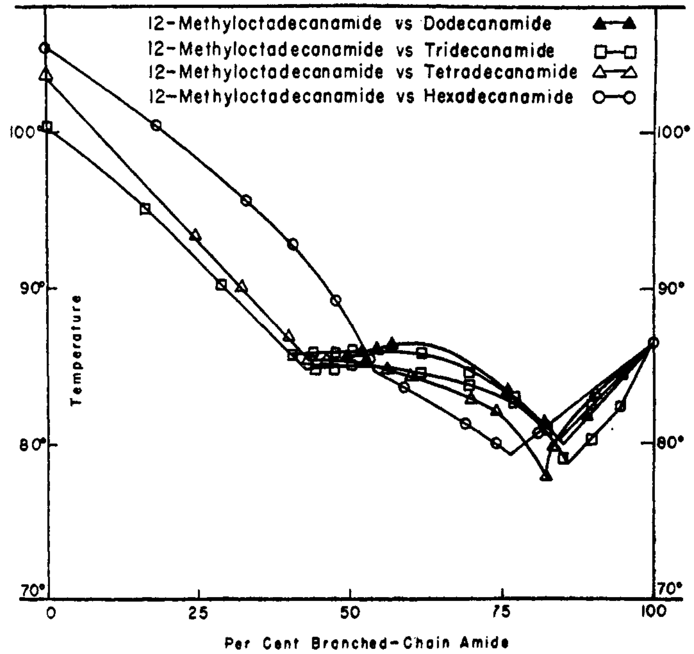

FIGURE 8

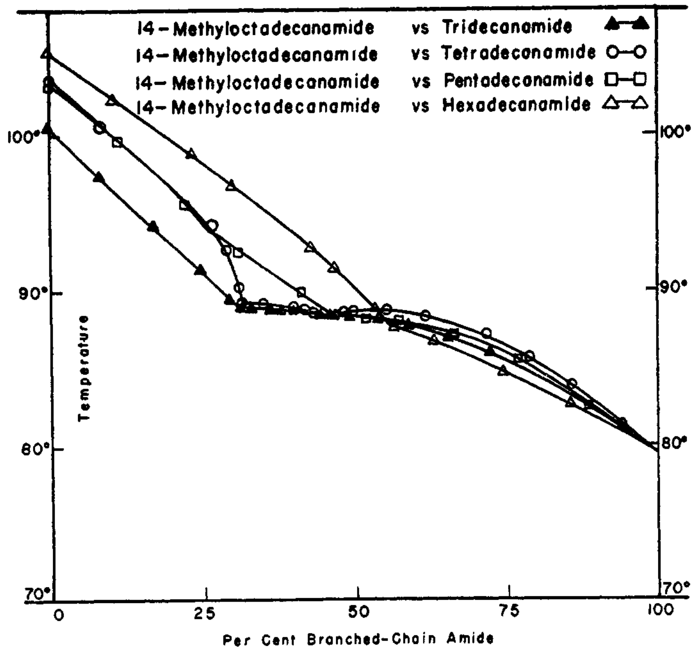

FIGURE 9

absorption spectra. There seems no probability of confusing the curves here presented. Even for adjacent positions, such as 10-, 11-, and 12-, the curves are quite different. The differences in the curves are due to molecular compound formation, and differences in solid solution, and these matters seem very sensitive to differences in the shapes of the molecules. The most similar curves obtained are those, in Figure 4, of hexadecanamide with 7-methyl- and 9-methyl-octadecanamide; however, careful inspection shows noticeable differences here. The curve for the 7-methyl isomer either has a barely perceptible eutectic at about 80%, or else it is the anomalous type shown for the *dl*-isomer in Figure 10. This feature is not present in the curve for the 9-methyl isomer. Also, the 9-methyl isomer shows a region of double melting points, and this was not detected for the 7-methyl isomer.

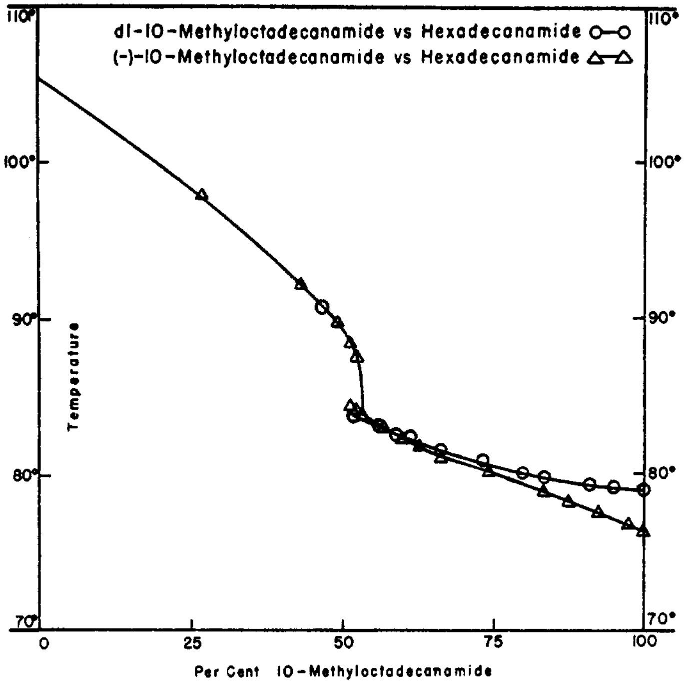

FIGURE 10

Effects of stereoisomerism. Since naturally-occurring acids or acids obtained by degradation of natural products are likely to be optical isomers, it is important to consider whether differences might arise with optical isomers. Weitkamp (3) compared the curves obtained with *dl*- and *d*-16-methyloctadecanoic acids, and they were nearly identical until there was reached a mixture containing about 95% branched-chain acid. Beyond this point, the curve for the *d*-acid continued to the origin at the melting point of this isomer, while the curve for the *dl*-isomer suddenly rose to the melting point of that isomer. We have verified this sharp break in the curve for the *dl*-isomer. This behavior was attributed to the complete dissociation of the *dl*-compound as soon as a few per cent of the normal acid was present to act as solvent. In Figure 4, the curve for 5-methyloctadecanamide and hexadecanamide shows a similar behavior, and the lower curve with tridecanamide in Figure 8 also has a eutectic near 95%. Thus, in these instances, stereoisomerism introduces no serious additional complexity.

In one instance, 10-methyloctadecanamide, a very unusual type of curve was obtained with the *dl*-isomer, as shown in Figure 10. When this curve was plotted it was realized that it was irregular, in that the portion between the eutectic and 100% branched-chain amide was bowed down instead of up, as was the case with all other curves which have been determined. Fortunately the *d*-isomer was available (4) for comparison, and determination of the curve with this isomer shows that this appears to be an unusual case in which the *dl*-compound gradually dissociates more completely into the *d*- and *l*- components, as its solution in the normal acid becomes more dilute. The two curves do not become coincident until the concentration of branched-chain amide has been reduced to about 60%. The curve for the *d*-isomer has a barely detectable, but definite, second eutectic at about 65%. In the curve for the *dl*-isomer, this eutectic is obscured by the gradual dissociation. It is noteworthy that, even in this unusual instance, the shape of this curve can hardly be confused with any of those in Figures 6–9.

Discussion of the scope of the method. It is apparent that with the data here presented, the method can be applied only to monomethyl acids. Until more data are obtained, the value of the method cannot be evaluated for acids with larger branching groups or multiple branches. However, most alkyl substituents occurring in nature are methyl, and the number of branches may be estimated by use of the data of Ginger (5), who has adapted the Kuhn-Roth method to use with high molecular weight branched-chain acids.

There is no certainty that the types of curves obtained in the methyloctadecanoic acid series will hold for acids of different molecular weight, and investigation of acids of different molecular weight is indicated as the next step in extending this method. It seems reasonable that the relationship of the branching methyl to carboxyl is the most important structural relationship, and that a few carbons more or less on the end of the chain would exert no great effect in molecules of these molecular weights. Several interrelationships between acids of different molecular weights have been mentioned in the preceding paper (2) of this series.

EXPERIMENTAL

Method and apparatus. The melting points of the mixtures were determined in tubes of about 5-mm. diameter, with walls of about 0.2-mm. thickness. A sample of 50–100 mg. (weighed accurately to 0.1 mg.) of one component was weighed in the tube and the m.p. determined as the temperature at which the last crystalline material was no longer visible. The material was stirred with a No. 18 tantalum wire during all observations of m.p. A suitable increment of the other component was weighed in a tube similar to the long-handled weighing tubes used in microanalysis (6). The melting-point tube (containing the solidified sample) was inverted over the weighing tube, the weighing tube was raised until its end was close to the solidified sample in the melting-point tube, and the tubes were inverted and the increment shaken into the melting-point tube. The weighing tube was then removed and weighed to give the weight of the increment. This technique avoided getting material on the upper parts of the wall of the melting-point tube. The new mixture was melted and stirred for several minutes with the tantalum wire; then the m.p. was determined. Another increment was then added, and the process was repeated until the mixture contained more than 50% of the component being added. This process was then repeated, starting with a sample of the other component, and the curve extended until it overlapped that determined from the other direction. In any instances where the curves from the two directions did not fit, re-determinations were made, and in places where a doubtful eutectic developed, additional points were added in that region.

The melting-point tubes were heated in an apparatus similar to the Hershberg melting point outfit (7), except that our apparatus is larger. The front tube of the apparatus is 2.4 cm. in diameter and 29 cm. high. With this larger apparatus, there was no difficulty in holding the temperture constant to ±0.1° for ten minutes or longer, but the temperature could be varied rapidly when desirable. Melting points were observed through a reading glass and a supplementary hand lens. After the melt had been stirred thoroughly, it was removed from the bath and cooled with stirring until crystallization began, then returned to the bath at a temperature below the m.p. The bath was raised slowly until melting occurred, then the temperature was lowered 0.1°, the melting point tube was removed until crystallization set in, then returned to the bath and stirred for about ten minutes or until the mixture melted. This process was repeated until it was observed that melting occurred at a given temperature and did not occur 0.1° below this temperature. Thermometers were of the Anschütz variety and were standardized against thermometers calibrated by the Bureau of Standards.

Melting points determined in this manner were easily reproducible to 0.1°, and the error in weighing increments of sample introduced a greater variation than the error of observing m.p., in regions where the m.p. changed rapidly with composition.

The greatest uncertainty exists in regions of double melting points. The behavior in these regions varied, but usually, as the composition was varied, one m.p. became less distinct as the other became more distinct. Often, there was a transition region where neither m.p. was distinct. In some cases, the lower m.p. was obtained unless the mixture was cooled considerably below this point before being returned to the bath, and sometimes standing overnight was necessary before the higher m.p. was observed. In other cases, the mixture melted at the lower m.p., then partially resolidified and re-melted at the higher point. When the higher form began to separate before the lower was completely melted the lower m.p. became rather inaccurate. In the most dubious cases it was not recorded. It will be noted that the double melting points are especially common when the branching group is near the middle of the chain. In some of these regions of double melting points, the curves also become so steep that certain regions are in doubt, for a change of a few per cent in composition changes the m.p. by several degrees.

SUMMARY

There has been determined a series of melting-point curves for binary mixtures of branched and normal acids and amides. These curves have been analyzed and used as the basis for a method of locating a branching methyl group at any position along the chain of a fatty acid. The probable scope and limitations of the method are estimated.

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