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# LOCATION OF A BRANCH IN A SATURATED CARBON CHAIN*

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**Abstract**—Oxidative degradation by chromic acid in glacial acetic acid has been developed as an effective method for location of a branch in a saturated carbon chain. Detection of cleavage products by use of gas phase chromatography allows convenient application of the method to samples of 3–10 mg. Several branched-chain acids and a branched-chain hydrocarbon have been found to yield the expected cleavage products; however, selective attack on a branch located *alpha*, *beta* or *gamma* to carboxyl is not significant. The mechanism of the oxidation and the protective influence of carboxyl are discussed.

ALTHOUGH there are available to the organic chemist numerous methods for stepwise degradation of a carbon chain *via* attack at a multiple linkage or a polar substituent group, in most instances the chain is degraded by only one or two carbon atoms per operation. In instances where a branch in a chain is located remotely from a polar functional group, stepwise degradation until the branch is reached becomes laborious and is likely to require relatively large amounts of starting material. Our attention has been directed to this problem as a result of our investigations of the branched-chain acids from tubercle bacillus, notably the physiologically active $C_{27}$-phthienoic acid.[1] Structural features near carboxyl and the adjacent double bond were elucidated[2] by investigation of rather small amounts of material, largely by utilization of analytical and physical measurements; however, subsequent investigations[3] have indicated only a strictly tentative structure for the branching group which is more remote from carboxyl than the 4-position.[4] Thus, degradative methods designed to directly attack the position of branching in an alkane chain have come under investigation.

Among the processes in which a tertiary position is selectively attacked under appropriate conditions are halogenation, nitration and sulfonation; however, these methods suffer the common disadvantage that additional steps are necessary in order to eventually break the chain and give products whose identification will reveal the structure of the substance degraded. In contrast, several methods of oxidation not

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[1] J. Cason and G. Sumrell, *J. Biol. Chem.* **192**, 405 (1951); H. Husseini and S. Elberg, *Amer. Rev. Tuberc.* **65**, 655 (1952).

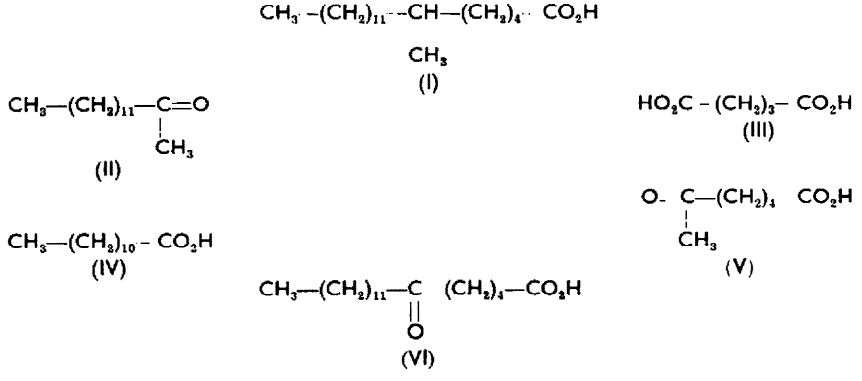
[2] J. Cason, N. K. Freeman and G. Sumrell, *J. Biol. Chem.* **192**, 415 (1951); J. Cason and C. F. Allen, *Ibid.* **205**, 449 (1953).

[3] J. Cason, H.-R. Urscheler and C. F. Allen, *J. Org. Chem.* **22**, 1284 (1957).

[4] Investigations carried out at the Oxford laboratories, based originally on degradation of a mixture of higher fatty acids from the tubercle bacillus, have resulted in assignment of the structure, 2,4,6-trimethyl-2-tetracosenoic acid, to a component acid termed mycolypenic acid [cf. D. J. Millin and N. Polgar, *J. Chem. Soc.* 1902 (1958) and references cited therein]; however, the complexity of the mixture of $\alpha,\beta$-unsaturated acids in the tubercle bacillus is such that the identity of $C_{27}$-phthenoic acid and mycolypenic acid (cf. ref. 3) can hardly be regarded as established. Thus, degradation of $C_{27}$-phthienoic acid is being pursued actively, as is separation of other components from the mixture.

only attack a tertiary hydrogen rapidly but also cleave the chain. Although ozone has been reported[5] to react "primarily at the tertiary carbon atoms", in the present investigation the rate of reaction was found to be quite slow, and the yield of cleavage products was very small. Oxidation with alkaline permanganate or with sodium dichromate in acetic acid appeared insufficiently selective to be of interest; however, oxidation with chromic acid in acetic acid[6] has proved quite useful, and utilization of gas phase chromatography for detection of cleavage products renders the method effective for degradation of quantities of 5 mg or less.

In order to determine if the reaction conditions employed by Spielman[6] could be improved, the oxidation of 6-methyloctadecanoic acid (I) was examined. Expected primary cleavage products are those represented by formulas II–VI. In this oxidation, as well as others examined, the keto acid corresponding to VI was obtained in such small amount that it was rarely detected at all. This point is of interest in connection with the mechanism of the oxidation to be developed subsequently. For evaluation of the effectiveness of conditions, the yield of ketone II was determined. It is the




only neutral primary degradation product and appeared as the sole band in gas chromatography of the neutral product. The more pertinent results are summarized in Table 1. The conditions recommended by Spielman (similar to Run 1) were found to employ a favorable ratio of oxidizing agent, in that most of the starting acid was consumed (cf. footnote *b*) and increase in oxidizing agent lowered the yield of 2-tetradecanone (Run 2). Optimum conditions appear to involve dissolution of the reactants before heating and use of a rather dilute solution (Runs 3–5); therefore, the conditions represented by Run 5 have been adopted as those preferred for the degradation.

The expected cleavage products have also been obtained from 9-methyloctadecanoic acid and from 10-methyloctadecanoic acid (VII). From 2,5-dimethyloctadecanoic acid, there were obtained only the cleavage products resulting from the branch at the 5-position; therefore it is indicated that carboxyl stabilizes the adjacent position against oxidative attack. Further investigation of the stabilizing influence of carboxyl is discussed below. Representative recorder tracings obtained from gas chromatography of degradation products are illustrated in Fig. 1. The two lower curves are

[5] J. H. Durland and H. Adkins, *J. Amer. Chem. Soc.* **61**, 429 (1939).

[6] This method of oxidation was used on a relatively large scale by M. A. Spielman, *J. Biol. Chem.* **106**, 87 (1934), to establish the structure of tuberculostearic acid as 10-methyloctadecanoic acid. From 2 g of tuberculostearic acid were obtained 30 mg of the semicarbazone of 2-decanone and 30–70 mg of azelaic acid.

TABLE 1. OXIDATION OF 300 mg OF 6-METHYLOCTADECANOIC ACID

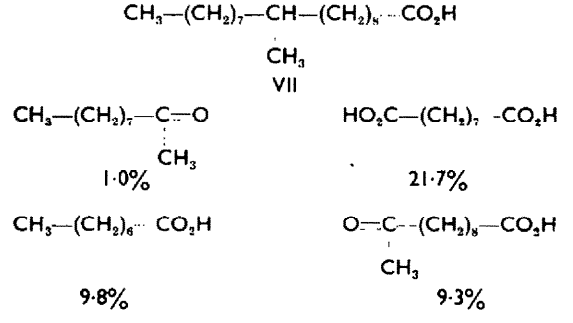
Temp: 60–70°. Time: 2–3 hr 6 moles of $CrO_3$ per mole of acid, unless otherwise indicated.

| Run No. | Method of $CrO_3$ addition | ml of HOAc | Yield of Neutral (mg) | Yield of 2-Tetradecanone (mg) |
|---|---|---|---|---|
| 1 | solid to hot solution | 7·5 | 12 | 4 |
| 2[a] | solid to hot solution | 7·5 | 22 | 2·5 |
| 3 | solution to hot solution | 18 | 20 | 5 |
| 4 | dissolved in cold solution | 22 | 21 | 8·5 |
| 5[b] | dissolved in cold solution | 50 | 24 | 9·5 |

[a]In this run, there were used 9 moles of $CrO_3$ per mole of acid.
[b]In this run, there were also detected 18 mg of dodecanoic acid and 30 mg of the starting acid (by gas chromatography of the esters).

characteristic ones, whereas the upper curve shows the largest secondary bands that have been encountered.

Weight per cent yields were rather carefully determined for the four principal degradation products of acid VII, and these values are recorded beneath the formulae for these compounds.




The total yield of keto acid and dibasic acid, resulting from the two dominant cleavages at the tertiary carbon, amounts to about 48 per cent on a molar basis. Since there are present in the molecule thirty secondary hydrogen atoms and six primary hydrogen atoms, and the primary degradation products contain many secondary hydrogen atoms, a highly selective attack at the tertiary hydrogen is demonstrated.

It may be noted that the keto acid and monobasic acid from oxidation of acid VII are obtained in similar yields, as would be expected, since these products result from cleavage of the bond to the tertiary carbon on the side remote from carboxyl. In contrast, the ketone and dibasic acid resulting from cleavage in the alternate position at the tertiary carbon were detected in a ratio of about 1 : 22. Furthermore, the yield of dibasic acid is more than twice that of the monobasic acids. These data clearly indicate that carboxyl has a significant stabilizing effect against attack by the oxidizing agent, and the magnitude of the effect suggests that the protection afforded by carboxyl extends beyond the *alpha* position.

The data in Table 2 show that the protecting effect of carboxyl extends as far as the *gamma* position, at which point it ceases rather abruptly. Yields of 2-tetradecanone

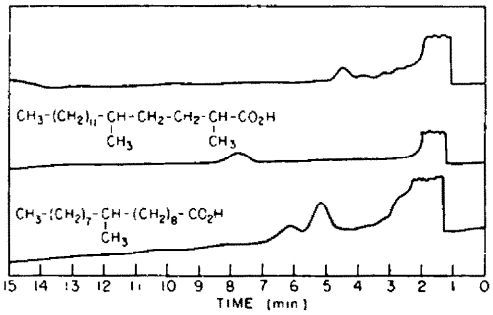



FIG. 1. Recorder tracings from gas chromatography of oxidative degradation products. There was used a 3 m × 15 mm o.d. column of Pyrex glass containing as partitioning agent high vacuum silicone grease dispersed on 30–60 mesh Celite fire-brick (4 parts grease : 10 parts fire-brick); helium pressure was 18·5–22 cm of mercury; flow rate was 150–180 ml/min; temp for upper and lower tracings was 280°, for center tracing 260°. The initial off-scale band in each tracing is due to the benzene used as solvent for the material injected.

*Lower curve*: Esters of acids from degradation of 10-methyloctadecanoic acid. Retention times for major bands were 2 : 40 (min, sec) for methyl octanoate, 5 : 10 for dimethyl azelate, and 6 : 07 for methyl 10-oxohendecanoate. For quantitative determination of octanoate, its band was resolved from the solvent band by chromatography at lower temperature.

*Center curve*: Neutral material from degradation of 2,5-dimethylheptadecanoate; retention time for 2-tetradecanone was 7 : 45.

*Upper curve*: Esters of acids from degradation of 2,5-dimethylheptadecanoate. Retention time for methyl dodecanoate was 4 : 50. Area under the largest secondary band (at 3 : 45) is about one-fifth that of the dodecanoate band. At the edge of the figure, the band for ester of starting acid is developing.

and dodecanoic acid from 2,5-dimethylheptadecanoic acid are about half the yields of cleavage products from the 10-methyl acid, and the yields from the 6-methyl acid (cf. Table 1) are as good as those from the 10-methyl acid. The near normal yield of ketone obtained from the $\alpha,\beta$-unsaturated acid (last entry in Table 2) is of interest in connection with the mechanism of the oxidation. This yield also indicates that the

TABLE 2. DEGRADATION OF ACIDS WITH BRANCHES NEAR CARBOXYL

| Acid oxidized | Yield (wt. %) of degradation products[a] | |
|---|---|---|
| | Ketone | Acid |
| 10-Methyloctadecanoic | 1·0 | 9·8 |
| 2-Methyloctadecanoic | 0·2 | nil |
| 3-Methyloctadecanoic | *b* | *b* |
| 4-Methyloctadecanoic | *b* | *b* |
| 2,4-Dimethyldocosanoic | *b* | *b* |
| 2,5-Dimethylheptadecanoic | 0·4 | 4·7 |
| 2,4-Dimethyl-2-docosenoic | 0·6 | 0·05[c] |

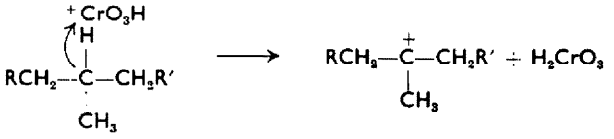
[a] The ketone and the ester of the acid from the terminal portion of the molecule were determined by gas chromatography. No effort was made to isolate the lower molecular weight fragments from the carboxyl end of the acid degraded. Thus, expected degradation products from 3-methyloctadecanoic acid would be 2-heptadecanone and pentadecanoic acid.

[b] Many small peaks in gas chromatography tracing, with no dominant one.

[c] In this degradation, there was also detected 6·3 % yield of 2-methyleicosanoic acid, from cleavage at the double bond.

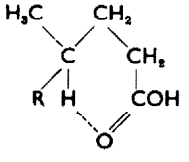
tertiary 4-position is attacked about as rapidly as the double bond; otherwise, the yield of ketone could hardly be greater than that from the 2-methyl acid.

In considering a mechanism for the oxidation, cognizance must be taken, not only of the data secured in the present investigation, but also of the considerable evidence supporting the thesis[7] that oxidations in acid solution are ionic in character and the attacking species in such oxidations is positive. In a solution of chromic anhydride in acetic acid, the most probable oxidizing species[7] is $^{+}CrO_3H$. Since Bartlett *et al.*[8] have shown that a carbonium ion very rapidly removes a hydride ion from the tertiary position in isopentane, the initial step in the presently investigated oxidation may be reasonably formulated as follows:



The higher electron density at the tertiary hydrogen, as well as the greater stability of the tertiary carbonium ion, would combine to promote the selective attack that is observed at the tertiary position. Loss of a proton by the initially formed carbonium ion would lead to a mixture of alkenes, with the higher energy methylene isomer in smaller amount. Subsequent oxidation of the alkenes would lead to the observed degradation products, with a smaller amount of the keto acid resulting from elimination of methyl.

If this proposed mechanism for the oxidation be correct, then an electron-withdrawing group such as carboxyl should indeed reduce the reactivity of hydrogens on the adjacent *alpha* position; however, such a direct inductive effect should drop off rapidly with distance and become negligible at the *gamma* position. The experimental observation that the hydrogens at the *gamma* position are no more reactive than those at the *alpha* position indicates an interaction between carboxyl and hydrogen at the *gamma* position. Several properties of fatty acids and derivatives have been successfully correlated[9] on the basis of a quasi ring conformation resulting from hydrogen bonding of the *gamma* hydrogen with carboxyl oxygen. For a $\gamma$-methyl acid, this ring would take the form of VIII, wherein the tertiary hydrogen should be

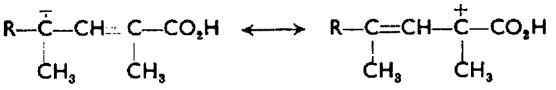


VIII

protected from attack by the oxidizing agent. Since hydrogens in the *beta* position are also relatively inert to oxidative attack, it is suggested that the inductive effect of carboxyl is an important factor in stabilizing the positions included in the quasi ring. Furthermore, the slightly reduced reactivity of hydrogens at the *delta* position may well result from the proximity of this position to carboxyl, in the quasi ring structure.

[7] L. S. Levitt, *J. Org. Chem.* **20**, 1297 (1955).
[8] P. D. Bartlett, F. E. Condon and A. Schneider, *J. Amer. Chem. Soc.* **66**, 1531 (1944).
[9] J. Cason and G. Sumrell, *J. Org. Chem.* **16**, 1177 (1951); H. A. Smith and J. P. McReynolds, *J. Amer. Chem. Soc.* **61**, 1963 (1939).

If a double bond is introduced in the $\alpha,\beta$-position in a fatty acid, and it has the *trans* configuration, a quasi ring such as indicated in VIII cannot form and the $\gamma$-hydrogen should lose its protection. The 2,4-dimethyl-2-docosenoic acid, whose degradation is indicated in Table 2, has the *trans* configuration,[10] and it will be noted that a relatively good yield of ketone was obtained on oxidation, in contrast to the behavior of the saturated acid. Failure to obtain significant amounts of octadecanoic acid from degradation of the unsaturated acid would be expected because the carbonium ion proposed as the first intermediate in the oxidation would have double bond character at the $\beta,\gamma$-position. Pertinent resonance forms would be the following:



In order to verify the greater susceptibility to attack of hydrogens beyond the *gamma* position, certain dibasic acids have been subjected to the same conditions of oxidation that are used for degradation of branched-chain compounds. In the case of azelaic acid, $HO_2C—CH_2—CH_2—CH_2—CH_2—CH_2—CH_2—CH_2—CO_2H$, the center carbon is the only one beyond the *gamma* positions, hence dominant degradation products should be succinic and glutaric acids, resulting from cleavage on either side of the central carbon. As judged by gas phase chromatography, 84 per cent of the dibasic acid survived the oxidative treatment. The total area under the bands resulting from degradation products was divided as follows:[11] succinic, 33 per cent; glutaric, 39·5 per cent; adipic, 14·5 per cent; pimelic, 11·8 per cent; suberic, 1·2 per cent. Since the yield of adipic acid, which results from attack at the *beta* or *gamma* position, is scarcely more than one-third the yield of glutaric acid, it may be concluded that the prime source of glutaric and succinic acids is attack at the single *delta* position.

Oxidation of hendecanedioic acid, containing three carbon atoms beyond the *gamma* positions, was especially striking. The results of this oxidation are summarized in Table 3. It may be noted that the degradation products which may result from attack at the *delta* or *epsilon* position are detected in twice the quantity of those resulting from attack at the *gamma* or *delta* position. Products resulting from attack at positions closer to carboxyl than *delta* have become insignificant.

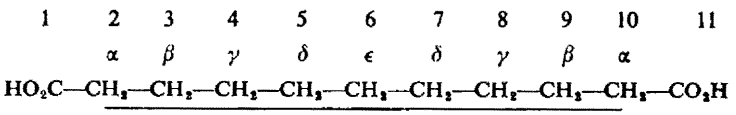
Oxidation of a normal monobasic acid is not subject to the selectivity observed for higher dibasic acids, for any hydrogens beyond the *gamma* position are equally subject to attack. This was observed experimentally by oxidation of dodecanoic acid. Bands of similar size were observed for the homologs from succinate (and heptanoate) to suberate. Recovery of dodecanoic acid was 75 per cent. Azelate was under the very large dodecanoate band and sebacate was on the trailing edge of it.

One aspect of the results obtained by degradation of normal dibasic acids is of importance in connection with interpretation of the results of degradation of higher molecular weight branched-chain compounds. If a branch or branches are so located that a primary degradation product is a normal dibasic acid with nine or more carbons,

[10] J. Cason and M. J. Kalm, *J. Org. Chem.* **19**, 1947 (1954).

[11] The ratio of moles of acids will be slightly more favorable to the lower molecular weight acids than the ratio of areas; however, this correction is small enough to be of no significance to the present discussion. It has been reported [R. H. Eastman, *J. Amer. Chem. Soc.* **79**, 4243 (1957)] that the weight ratio of two components is equal to the product of the ratio of areas under the bands and the ratio of the square root of the respective molecular weights. Thus, the correction factor for molar ratios would be the inverse ratio of square root of molecular weights.

TABLE 3. OXIDATIVE DEGRADATION OF HENDECANEDIOIC ACID




| Acid from degradation | Position attacked | Per cent of total area[a] |
|---|---|---|
| glutaric ($C_5$) | $\delta$ or $\epsilon$ | 28 |
| adipic ($C_6$) | $\delta$ or $\epsilon$ | 34·5 |
| succinic ($C_4$) | $\gamma$ or $\delta$ | 12 |
| pimelic ($C_7$) | $\gamma$ or $\delta$ | 18 |
| suberic ($C_8$) | $\beta$ or $\gamma$ | 3 |
| azelaic ($C_9$) | $\alpha$ or $\beta$ | 3 |
| sebacic ($C_{10}$) | $\alpha$ | 1·5 |

[a] Data recorded are referred to the total area ascribed to degradation products, which was 23 per cent of total area under all bands; the remaining 77 per cent was under the band due to recovered hendecanedioic acid. For relationship of molar ratio to area ratio, refer to footnote 11.

the small amount of further degradation of this product becomes selective, as has been described above. For example, if azelaic acid is a primary degradation product, further degradation of this dibasic acid yields principally succinic and glutaric acids. The gas chromatography bands for esters of these acids then become larger than bands from the other secondary degradation products, and there may arise the question that these bands represent monobasic esters (see below) from a primary degradation or products arising from impurities present in small amounts. This uncertainty may usually be resolved by consideration of the degradation expected from the dibasic acid obtained as a major chromatography band, and noting the ratio of the major band to the larger satellite bands.

Another factor of importance in interpreting gas chromatography of degradation products on silicone grease is the coincidence of bands due to dibasic esters and monobasic esters. Dimethyl succinate coincides with methyl heptanoate, glutarate with octanoate, etc. This factor rarely causes uncertainty because of the relationship of ketone to monobasic acid and keto acid to dibasic acid. If difficulty does arise in complicated degradation patterns, or from selective degradation of a dibasic acid, the distinction between monobasic and dibasic acids may be made by comparison of retention times for methyl and ethyl esters. The shift in retention time in going from methyl to ethyl esters will obviously be greater for the dibasic acid. In cases of remaining uncertainty, butyl esters may be invoked. Furthermore, chromatography on the partitioning agent known as Reoplex-400 (Geigy) shifts the dibasic esters to longer retention times in relation to the monobasic esters.

Since vigorous oxidation of other aliphatic structures leads to acids, it may be assumed that the presently investigated method is applicable to degradation of branched aliphatic structures containing various functional groups. As a confirmation of the utility of the method for degradation of saturated hydrocarbons, oxidation of the symmetrically branched alkane, 9,14-dimethyldocosane, $C_8H_{17}$—$CH(CH_3)$—$(CH_2)_4$—$CH(CH_3)$—$C_8H_{17}$, was examined. Major chromatography bands were

observed for three of the four products resulting from a single cleavage of the chain at the tertiary position: octanoate, decanone, and 5-methyl-tridecanoate (retention time between normal tridecanoate and normal tetradecanoate). The band for 7-methyl-2-pentadecanone was not observed (and hardly expected since a normal ketone is obtained only in low yield). A smaller band was observed for succinate, formed by successive chain cleavages. Another smaller band at slightly longer retention time than octanoate was probably due to the $\delta$-ketocaproic acid. Thus, the structure of the disubstituted alkane could be deduced from the degradation products obtained from oxidation of a 22 mg sample. The three major bands were detected from oxidation of a 6·5 mg sample.

As an example of a more complicated structure, there was investigated the degradation of 4,8,12-trimethyloctadecanoic acid. As usual, no degradation products were observed from cleavage at the 4-position. From cleavage at the 8-position, there were observed in the complicated chromatogram from degradation esters strong bands corresponding to 4-methylpimelic acid and to 4-methyl-8-oxononanoic acid. There was also observed in the neutral fraction a strong band corresponding to 6-methyl-2-dodecanone (slightly shorter retention time than a normal tridecanone). From cleavage at the 12-position, bands were observed corresponding to 2-octanone and hexanoic acid; however, these bands were weaker than normally obtained from primary degradation products. Several minor bands were probably due to products of successive cleavages; however, these could not be reliably distinguished from bands due to secondary degradation products. This latter development, also observed in the degradation of 9,14-dimethyldocosane, may well be an advantage in degradation of complicated molecules, for it simplifies detection of products from a single cleavage.

## EXPERIMENTAL

*Materials.* The branched-chain acids and the branched hydrocarbon used for oxidation were samples of compounds prepared previously in these laboratories in connection with the program of synthesis of branched-chain acids. Acetic acid used as solvent for the oxidations had been distilled from potassium permanganate. The chromic anhydride ($CrO_3$) used as oxidant was a commerical product.

*Gas phase chromatography.* The partitioning agent used in all chromatographic analyses was Dow-Corning high vacuum silicone grease, dispersed on 30–60 mesh Celite fire-brick or on the similar material marketed as "Chromosorb" (Johns–Manville). The ratio of grease to Celite was 4 : 10. For relatively large-scale runs (50–300 mg) in which yields of oxidation products were determined, there was used the rather large column described in connection with Fig. 1. For all runs on smaller samples, there was used a 1·5 m × 8 mm o.d. Pyrex glass column for chromatography at temp of 170–290°, and a similar 2 m column for temp below 170°. Thermal detection cells utilizing a thermistor were employed. Limit of detection was 1–50 $\mu$g, depending on the temp; below 200°, limit of detection was $<2$ $\mu$g.

In most instances, identification of degradation products rested on coincidence of the retention time with that of a known sample, although 2-tetradecanone from oxidation of 6-methyloctadecanoic acid was collected and found to have the I.R. spectrum of a ketone. Since neutral and acidic products were separated before chromatography, there seems no possibility of uncertainty concerning the identity of products giving major bands (distinction between di-esters and mono-esters has been discussed earlier). In addition, degradation of numerous compounds with a single branch in a known position has yielded no major bands except those corresponding to expected degradation products.

On each occasion that degradation products were chromatographed, known compounds were chromatographed in sequence; however, it is not necessary that the known compounds be identical with the degradation products. Three or more homologs of the degradation products, injected as a mixture, may be used to locate retention times at least as accurately as may be accomplished by

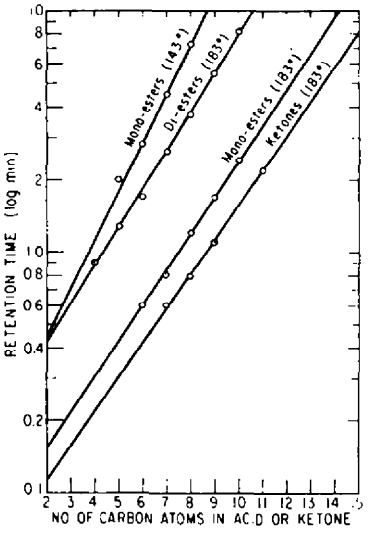



FIG. 2. Retention times in gas chromatography of homologous series. The essentially parallel lines for the different homologous series are characteristic, as is the increased slope at lower temp. At 183°, in sequential runs, retention time for methyl 10-oxohendecanoate was 7·8 min.

injection of only the known compounds. Sample semi-log plots used for location of retention times are presented in Fig. 2. Compounds with a branched chain, as encountered in degradation of 4,8,12-trimethyloctadecanoic acid, may be located with sufficient accuracy by reference to such plots. In the range of 8–20 carbon atoms, a compound with a single small branch falls between its normal isomer and the next lower homolog, usually about midway between. In tests of $C_{18}$ normal ketones, the retention time was found to decrease slightly as the keto progressed from the 2-position to the center of the chain, with the maximum shift no more than one-third the contribution of a methylene group. A keto ester is located at a very slightly shorter retention time than the di-ester of a dibasic acid with one less carbon (a sample of synthetic methyl 10-oxohendecanoate falls at a slightly shorter retention time than dimethyl sebacate). It follows that, even with a methyl branch, the di-ester and keto ester obtained by degradation of a branched-chain acid are readily separable and recognizable by the chromatography procedure.

Carboxylic acids may be chromatographed on the silicone grease partitioning agent; however, esters give stronger signals with a thermal detector, as well as sharper, more easily resolved bands.

*Procedure for oxidative degradation.* There is described the procedure regarded as the best of the various modifications examined. It provides for recovery of rather water-soluble products (such as glutaric acid), as well as rather volatile components (such as butanone).

A 10 mg sample of methyl 10-methyloctadecanoate (or the free acid) was added to a solution of 20 mg of chromic anhydride in 1 ml of glacial acetic acid. The resultant solution was heated under a water-cooled reflux condenser for 2–3 hr in a bath maintained at 65–70°. If a branched-chain compound is being oxidized, the initially red-brown solution becomes green after about 1 hr. To the cooled reaction mixture there was added 5% KOH until phenolphthalein indicator was turned pink (about 20 ml of alkali required). The resultant alkaline solution was continuously extracted with about 40 ml of ether for several hours. After the ether extract had been dried over anhydrous magnesium sulfate solvent was distilled through a half-meter column of the simple Podbielniak design.[12] A 10 ml pointed flask was used for the final stages of the distillation so that the residue might be brought nearly to dryness, then washed down with ether or benzene to about 0·2 ml volume in the tip of the flask. This concentrated solution was injected for gas chromatography of the neutral product. A volume of 10–50 $\mu$l is usually a satisfactory amount for injection.

[12] A satisfactory column is similar to that which has been described in detail by J. Cason and H. Rapoport in *Laboratory Text in Organic Chemistry* pp. 237–243. Prentice-Hall, Englewood Cliffs (1950). For present purposes, a heated head and jacket are unnecessary.

The alkaline solution, from which neutral material had been extracted, was concentrated to dryness under reduced pressure, and the residual salt was dried at 100° and 10–20 mm pressure for about 10 min. To the dry salts was added 10 ml of anhydrous methanol containing 20% by weight of conc $H_2SO_4$. This mixture was heated under a water-cooled reflux condenser in a bath at 45–50° for 3 hr, during which period there was added in three increments a total of 1 ml of acetone dimethyl acetal (2,2-dimethoxypropane). The cooled reaction mixture was placed in a continuous extractor containing 20 ml of water and neutralized to pH 7 with 10% KOH. The esters were continuously extracted with about 50 ml of ether,[18] and the extract was dried and concentrated for chromatography as described for the ketone fraction.

If a compound contains more than one branching group it is probably an advantage to use a slightly larger ratio of oxidizing agent, but not in proportion to the number of branches. For 4,8,12-trimethyloctadecanoic acid, containing two branches susceptible to selective attack, the best run was one in which there was used 40 mg of chromic anhydride for 16·5 mg of the acid.

[18] Unless low-boiling esters are expected as degradation products, this extraction is accomplished more reliably with benzene. Ether extracts considerable water from the aqueous methanol solution.