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

Distribution of Acetic Acid Carbon in High Fatty Acids Synthesized from Acetic Acid by the Intact Mouse

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Carboxyl-labeled acetate was injected into mice which were sacrificed in 4 hours and the resulting labeled fatty acids isolated. The acids were separated into saturated and unsaturated types and the latter hydrogenated. The pure acid components of the mixture were obtained by "amplified distillation" of the methyl esters. Each pure acid was degraded, one carbon at a time, by conversion to the phenyl ketone, oximination with a nitrite ester, cleavage of the oximino ketone with tosyl chloride and the resulting benzoic acid and aliphatic nitrile obtained. The process was repeated at least three times on each acid. It was found that the first and third carbons of the sixteen carbon atom acids were about equally labeled whereas in the eighteen carbon series the carboxyl carbon atom was about three times that of the third carbon atom. A small amount of activity was found in the even numbered atoms. From further degradation of one of the acids, it appears that the tail end of the molecule is not equally labeled. A discussion of these results is given.

The role of acetic acid and closely related two-carbon units in the biological formation of the long chain fatty acids has been extensively investigated[2-5] and it has been demonstrated that this synthesis involves the successive addition of a two-carbon unit by the reaction of the methyl group of the two-carbon unit with the carboxyl carbon atom of an acid containing two or more carbon atoms.[4-8] Most of the previous work has emphasized the fact that when a carboxyl-labeled acetic acid or similar fragment is utilized in such a synthesis the activity is distributed equally throughout the chain at alternate positions. Since the amounts of the shorter chain fatty acids normally present in the animal body are small, their involvement in this process would not play an important role. The over-all result of such a synthesis would lead to an approximately equal distribution of activity throughout the chain of fatty acids of sixteen or fewer carbon atoms. Such would not necessarily be the case for the eighteen carbon acids and, indeed, Zabin[4] has recently shown that the carboxyl end of such acids is more highly labeled when obtained from a short duration experiment. Such a result can be compared to the oxidation of long chain fatty acids where it has been found[9] that the rate of oxidation to carbon dioxide of the individual carbon atoms in palmitic acid is about equal whereas the carboxyl end of stearic acid is more rapidly β-oxidized than the residual sixteen carbon atoms. In the present investigation, we should like to report a more detailed analysis of the synthetic reactions involved in the formation of the long chain fatty acids and to demonstrate an apparent uniformity of mechanism in the synthesis of both the unsaturated and saturated fatty acids.

In order to conduct such a study, the separation of the pure acids from the body mixture must be achieved and in the earlier work[4,6-8] this has been accomplished by distillation of the esters of the acids in a micro-column. Such a procedure either demanded high dilution of the biosynthesized esters with unlabeled materials or many distillations or both in order to obtain pure acids. Both of these disadvantages may be overcome by use of the very effective oil-diluted ("amplified") distillation technique of Weitkamp[10] for the separation of small quantities of esters in an efficient macro-column. In such a process, the addition of a neutral, continuous boiling oil serves to keep the column full at all times and thus allows for proper and efficient operation with amounts of esters which would not be sufficient when employed alone.

Our procedure was to subcutaneously inject carboxyl-labeled sodium acetate into mice and to separate the crude fatty acids, as isolated from the whole mouse, into saturated and unsaturated fractions by the lead salt technique as modified by Schoenheimer and Rittenberg.[11] The unsaturated fraction was hydrogenated over platinum and the resulting saturated acids esterified. The esters then were distilled with oil in a 30-plate efficient column of the type described by Mitchell and O'Gorman,[12] the various fractions saponified and the acids isolated. The results of one such distillation are shown on Fig. 1. By this procedure, pure fatty acids could be obtained in one distillation.

In order to establish more fully the alternation of labeled carbon atoms in the isolated fatty acids, the pure compounds must be degraded one carbon atom at a time and this process repeated at least three times. To accomplish this required a method of degradation which could be performed in good yield on a small scale, and which yielded a degradation product in such a form that it could easily be degraded again. It would also be desirable to be able to isolate the carbon atom lost in this process in a convenient manner. Various methods have been employed to degrade fatty acids. The decarboxylation by pyrolysis with iron has been used by Rittenberg and Bloch[2] and by Zabin.[4] It has been shown by Gurin,[3] however, that this method is subject to error. This latter worker found that when carboxyl-labeled octanoic acid was decarboxylated

(1) Upjohn Company Fellow, 1947-1949.

(2) D. Rittenberg and K. Bloch, *J. Biol. Chem.*, **160**, 417 (1945), and earlier papers.

(3) R. O. Brady and S. Gurin, *ibid.*, **186**, 461 (1950).

(4) I. Zabin, *ibid.*, **189**, 355 (1951).

(5) G. Popjak, T. H. French, G. D. Hunter and A. J. P. Martin, *Biochem. J.*, **48**, 612 (1951); G. D. Hunter and G. Popjak, *ibid.*, **48**, v (1951).

(6) H. S. Anker, *J. Biol. Chem.*, **194**, 177 (1952).

(7) D. Stetten, Jr., and R. Schoenheimer, *ibid.*, **133**, 329 (1940).

(8) A. Klem, *Hvalradets Skrifter, Norske Videnskaps-Akad. Oslo*, No. 27, 1 (1943).

(9) E. O. Weinman, I. L. Chaikoff, W. G. Dauben, M. Gee and C. Entenman, *J. Biol. Chem.*, **184**, 735 (1950); E. O. Weinman, I. L. Chaikoff and W. G. Dauben, *ibid.*, **191**, 523 (1951).

(10) A. W. Weitkamp, *J. Am. Oil Chemists' Soc.*, **24**, 236 (1947).

(11) R. Schoenheimer and D. Rittenberg, *J. Biol. Chem.*, **113**, 505 (1936).

(12) F. W. Mitchell, Jr., and J. M. O'Gorman, *Anal. Chem.*, **20**, 315 (1948).

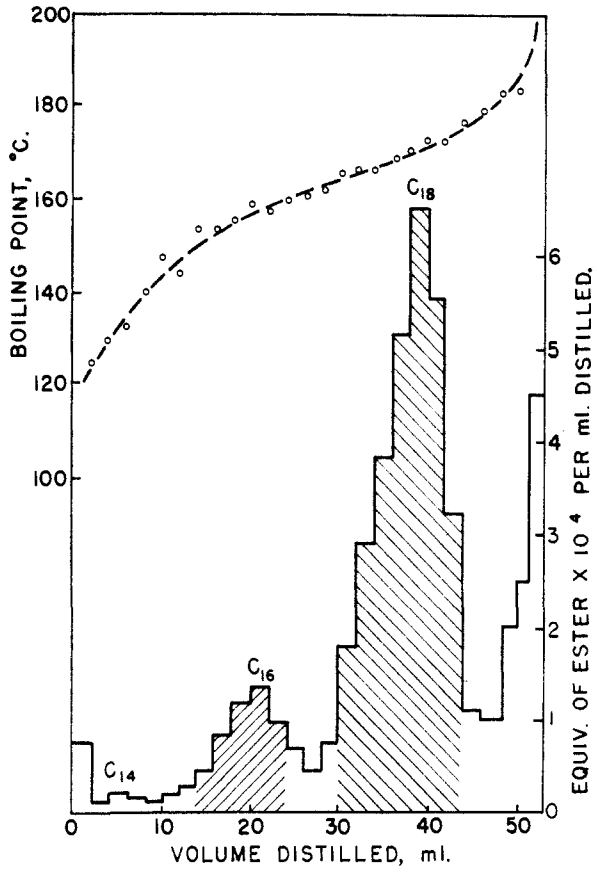
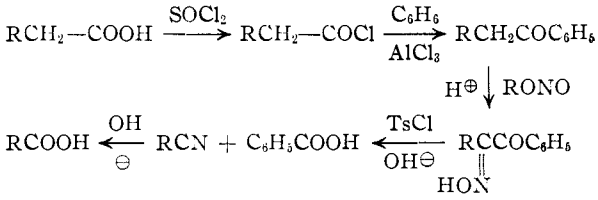


Fig. 1.

with iron, the specific activity of the carboxyl carbon atom was only 85% of theory; the cause of this discrepancy was not investigated. In addition to this dilution error, the decarboxylated product does not lend itself to further degradation. Barker,[13] working with the lower fatty acids, has employed the Barbier–Wieland method of degradation. When applied to the higher fatty acids, it has been found that the yields are only average and, in addition, the evolved carbon is isolated as the carbonyl group in benzophenone and thus dilutes the activity by a factor of thirteen. Ställberg-Stenhagen[14] has developed an excellent method, which was employed by Anker,[6] involving the decarboxylation of the silver salt of an acid with bromine to yield the next lower alkyl bromide (Hunsdiecker reaction). This halide is allowed to react with sodium acetate, the ester hydrolyzed and the resulting alcohol oxidized to an acid. This method proceeds in good yield and appears to be free from competing, contamination reaction. Anker,[6] nevertheless, has reported a lower specific activity of the carboxyl carbon than would be expected and believes this may be due to extraneous carbon dioxide. Recently, Phares[15] has found that the degradation of an acid to an amine with hydrazoic acid (Schmidt reaction), followed by oxidation of the amine gave rise to the next lower acid in good yield. As with the foregoing method, extraneous carbon dioxide can also arise. Popjak and his co-workers[5] have reported a scheme which degrades an acid two carbon atoms at a time. The evolved two-carbon unit is acetic acid which is, in turn, degraded *via* acetone and the hypoiodite reaction. Although the over-all yield is good, many manipulations are involved.

In the present work, the novel method of degradation involving a second-order Beckmann rearrangement of an α-diketone monoxime which was first utilized in the fatty acid series by Darzens and Mentzer[16] has been reinvestigated. The procedure developed was to convert the acid to the phenyl ketone by allowing its acid chloride to react with benzene and aluminum chloride under Friedel–Crafts conditions. The phenyl ketone was then nitrosated with a nitrite ester under acidic conditions and the resulting monoxime rearranged in the



presence of *p*-toluenesulfonyl chloride and alkali. The removed carbon atom is recovered as carboxyl-labeled benzoic acid which is easily obtained pure. The nitrile is hydrolyzed to the acid and the process can be repeated. The over-all yield from acid to acid was about 50–75% in most cases studied, and no extraneous carbon dioxide can be introduced into the process. The convenient isolation of benzoic acid removes the necessity of collecting carbon dioxide although the specific activity will be diluted by a factor of seven in benzoic acid. Such a procedure involves no strong oxidizing agents and requires no special apparatus.

The results obtained are given below and the isotope content of the different carbon atoms of the isolated palmitic and stearic acid was calculated as percent of the total activity of the acid.

	CH_3CH_2—$(CH_2CH_2)_5$—	—CH_2—	—CH_2—	—CH_2—	—COOH
Palmitic acid	66	2	12	1	13
	CH_3CH_2—$(CH_2CH_2)_6$—	—CH_2—	—CH_2—	—CH_2—	—COOH
Stearic acid	75	1	8	0.5	29

The unsaturated acids after hydrogenation, separation and degradation had the following isotopic distribution.

C_{16}	CH_3CH_2—$(CH_2CH_2)_5$—	—CH_2—	—CH_2—	—CH_2—	—COOH
	76	14	1	15	
C_{18}	CH_3CH_2—$(CH_2CH_2)_6$—	—CH_2—	—CH_2—	—CH_2—	—COOH
	67	10	2	23	

The approximate equal activity in the first and third carbon atoms of palmitic acid suggest that, in the main, palmitic acid is *totally* synthesized from two-carbon units and that only a very small quantity of it is formed by elongation of pre-existing high fatty acids, such as myristic. This latter

(13) E. R. Stadtman, T. C. Stadtman and H. A. Barker, *J. Biol. Chem.*, **178**, 677 (1949).

(14) S. Ställberg-Stenhagen, *Arkiv Kemi*, **1**, 153 (1949).

(15) E. F. Phares, *Arch. Biochem. Biophys.*, **33**, 173 (1951).

(16) G. Darzens and C. Mentzer, *Compt. rend.*, **213**, 268 (1941).

acid has been shown to be involved in such chain elongations[6] but no evaluation of its utilization has been possible. In contrast, the distribution of the isotope in stearic acid is quite different, the carboxyl carbon atom containing about three times the amount of carbon fourteen found in the third carbon atom. The residual sixteen carbon atoms of stearic acid (equivalent to palmitic acid) show an isotope distribution similar to that of palmitic acid. Such a distribution would indicate that a significant quantity of this eighteen carbon acid was formed from the sixteen carbon moiety. This finding is in agreement with the results of Stetten and Schoenheimer[7] which demonstrated that as much as 38% of the stearic acid was derived from depot palmitic acid in an eight-day experiment. This utilization of the normal fatty acids in fatty acid synthesis fits in quite nicely with the recent detailed postulate of Weinhouse[17] in which he assumed immediate formation of an activated fatty acid (a thiol ester with CoA) in fatty acid synthesis and thus not requiring the intermediate formation of the free fatty acid as such.

The results obtained from the degradation of the unsaturated acids show that the isotope distribution pattern is practically identical with that of the saturated acids. These results further substantiate the postulate of Anker[6] that the formation of the unsaturated acids may proceed through the intermediate saturated fatty acids. It still is possible, however, that the synthesis of the eighteen carbon unsaturated acids may proceed directly from the unsaturated sixteen carbon acid but, if so, at one stage they must be in rapid equilibrium with the saturated acid.

It would appear that the radioactivity in palmitic acid is distributed uniformly throughout the chain at alternate carbon atoms. For example, it will be noted that the first and third carbons of palmitic acid are about equally labeled. If one assumes that in the remaining twelve carbon atoms there are six intact two-carbon units, then, each unit has an average isotope concentration of 11%. This is to be compared to 13 and 12% for the first and third carbon atoms, respectively. Similar results are obtained in all four cases. This assumption was investigated in one case. It was found that when the residual acid from the degradation of the eighteen carbon unsaturated acids was oxidized with chromium trioxide and the acetic acid formed from the terminal two-carbon atoms isolated, only 4% of the over-all activity of the original eighteen carbon acid was present as compared to a calculated value of 9% of the activity if distributed equally throughout the chain. It must be concluded that the activity in the fatty acids formed in this short term experiment (4 hours) is not uniformly distributed throughout the chain.[18] A similar result has been reported by Anker[6] in his myristic acid feeding experiment. He found 25% of the total activity in carbon atoms 4 to 16 in the isolated palmitic acid. Again assuming six two-carbon units in this moiety, the average activity would be 4%; this value is to be compared with the experimental finding of 2% in the terminal two-carbon unit. The foregoing results are in contrast to those reported by Zabin[4] who found about equal distribution throughout the chain of palmitic acid.

A small but significant amount of activity was also found in the even-numbered carbon atoms. Although the method of degradation employed in the present work should preclude such activity as being due to contamination, due to the very low activities found, it cannot be stated with assurance that trace quantities of fatty acids were not present in the benzoic acid analyzed. A similar finding of activity in such carbon atoms has also been reported by Anker[6] and he also was not certain whether the results were due to the compounds themselves or to a contamination introduced in his degradation procedure. If, however, such activity is a real result there is, at present, no known pathway by which such labeling can occur. The $C_3 + C_1$ condensation to acetoacetate[19] could give rise to a carboxyl labeled two-carbon unit but no pathway exists for the transformation of such a labeled species to a methyl-labeled unit. An alternate mechanism involving the reaction of formate and glycine[20] to form serine, pyruvate and then a two-carbon unit is unlikely in view of the finding that carbon dioxide is not transformed to formate in liver slice.[21]

It is of interest to compare these results on the synthetic pattern of fatty acids from acetate with the reverse process, the oxidation of fatty acids. Work in this Laboratory has shown[9] that the primary fate of palmitic acid upon β-oxidation is complete oxidation to small units which can enter the tricarboxylic acid cycle and very little lauric or myristic acid is formed. With stearic acid a very similar pattern is obtained except for the fact that a measurable quantity of stearic acid is oxidized to palmitic acid which in turn has two pathways open to it, deposition and oxidation. Thus, it can be concluded that all of the carbon atoms of palmitic acid are of about equal activity in the oxidation reaction and that only a very small quantity of intermediate length acids are formed and utilized for other reactions. Since palmitic acid is present in the body it can act as a stable intermediate and as a building block for further synthesis. Thus if the reverse picture to oxidation is to be obtained in synthesis, one would expect about equal labeling throughout the chain of palmitic acid but with stearic acid, a greater portion would be found in the carboxyl carbon. This is the result found in present study.

Acknowledgment.—The authors wish to thank Professor I. L. Chaikoff and Dr. P. Srere of the Department of Physiology of this University for furnishing the labeled fatty acids employed in the work and wish to express their appreciation to the Upjohn Co. for kindly supporting this work.

(17) S. Weinhouse, *Arch. Biochem.*, **37**, 239 (1952).

(18) If a significant quantity of fatty acid were formed from preexisting four carbon units or if the acetyl-CoA formed from the terminal two carbon units of preexisting fatty acids differed from that formed from ingested acetate and acted predominately as an acetylation agent, then it might be expected that the residual two carbon unit as well as the adjacent unit might have quite different activities from the others.

(19) G. W. E. Plaut and H. A. Lardy, *J. Biol. Chem.*, **186**, 705 (1950).

(20) W. Sakami, *ibid.*, **176**, 995 (1948).

(21) P. Siekevitz and D. M. Greenberg, *ibid.*, **180**, 845 (1949).

Experimental[22]

Biological Conversion of Carboxyl-labeled Acetic Acid into High Fatty Acids.—This experiment was performed by Professor I. L. Chaikoff and Dr. P. Srere. Sodium acetate-1-C^{14} ($\sim$ 1 mc.) in isotonic saline solution was injected subcutaneously into 20 female mice weighing a total of 500 g. After 4 hours, the animals were sacrificed and the combined carcasses digested in a solution of 30% alcoholic potassium hydroxide. The resulting mixture was diluted with water, filtered and extracted with ether. The remaining aqueous solution was acidified and extracted with petroleum ether. Upon evaporation of the petroleum ether, a residue of 6.5 g. of crude fatty acids was obtained.

Separation of Unsaturated Acids from Saturated Acids.—The 6.5 g. of crude fatty acids were treated with lead acetate according to the procedure of Twitchell.[23] After recrystallization of the lead soaps, the fatty acids were regenerated with 4 *N* nitric acid and 1.6 g. of saturated fatty acids was obtained.

The filtrates from all separations of lead soaps were combined and were concentrated under reduced pressure to about 20 ml., diluted with water and acidified with 4 *N* nitric acid. After extraction with ether and evaporation of the solvent, 5.1 g. of crude unsaturated fatty acids was obtained. The contaminating saturated fatty acids were "washed-out" from the unsaturated acids by the method of Schoenheimer and Rittenberg[11] using pure palmitic and stearic acids. This process was repeated three times and the crude unsaturated acids amounted to 3.6 g. and they were hydrogenated immediately over platinum in methanol at room temperature.

Amplified Distillation of Fatty Acid Esters.—The two mixtures of fatty acids, saturated acids and unsaturated acids which had been hydrogenated, were esterified with methanol in the presence of 1% sulfuric acid. Each mixture of crude esters was then separated by fractional distillation at 5 mm. pressure in the presence of 15 to 20 times its weight of "Eureka White Oil."[24] The oil had previously been distilled and a fraction boiling from 120–230° (5 mm.) was used. The column packing was of the type described by Mitchell and O'Gorman.[12] The column had an internal diameter of 6 mm. and was 560 mm. in length. Fractions were taken in one or two-ml. portions and collected in small tubes. For a typical run see Fig. 1.

Isolation of Pure Fatty Acids.—The fractions of distillate were saponified with a solution of potassium hydroxide in isobutyl alcohol ($\sim$ 0.2 *N*). The tube receivers, each containing one or two ml. of ester–oil mixture, were placed individually into 500-ml. erlenmeyer flasks and 5 ml. of the alcoholic alkali added to each. The flasks were heated until the isobutyl alcohol refluxed, allowed to cool for 1–2 minutes and then the heating and cooling repeated. Using phenolphthalein indicator, each sample was titrated with 0.100 *N* hydrochloric acid. The titer of the alkali was determined with standard acid after an aliquot 5-ml. portion had been heated with 2 ml. of pure carrier oil in the same manner as the ester sample.

The concentration of each sample in terms of equivalents per ml. of distillate (when 2-ml. samples were used, the average value was plotted) was plotted against total ml. collected at the time the fraction was taken. A step-like plot then could be drawn to show the results of the distillation. From such graphs, one chooses which fractions to combine. In general, all fractions falling into one vertical band were combined. Only those fractions lying near or at the "minimum" were not used since they were likely to be mixtures. The material in these minima was recycled or discarded. In Fig. 1, it is illustrated which fractions were combined to obtain pure palmitic and stearic acids; all material in the shaded areas of one band was used.

After combining the appropriate fractions, the pure fatty acids were isolated from the saponification mixture. Since care must be taken that all of the carrier oil is removed, the isolation will be described in detail. Excess hydrochloric acid was added to the combined fractions, a small amount of hexane was added and the aqueous layer removed. The hexane solution was transferred to a large erlenmeyer flask and a solution of potassium hydroxide in methanol–water (2:1) added. The mixture was heated on a steam-bath for one hour to ensure complete conversion to the potassium salt which is soluble in the methanol–water mixture on cooling. In some cases, additional methanol must be added. The mixture was transferred to a separatory funnel with minimum mixing and without shaking the methanol–water layer was removed. The removed solution was rewarmed and extracted, by gentle swirling, with small volumes of hexane. The solution of the salts, free from oil, was diluted with water, the acids obtained by acidification and then recrystallized from acetone–water (3:1).[25] From the fractions of the saturated acids there was obtained 293 mg. of palmitic acid, m.p. 61–62°, and 173 mg. of stearic acid, m.p. 66–67°. From the hydrogenated unsaturated fraction there was obtained 102 mg. of palmitic acid, m.p. 61.0–61.5°, and 889 mg. of stearic, m.p. 67–68°.

Degradation of Fatty Acids.—The following procedure, described specifically for the conversion of palmitic acid (isolated from the unsaturated fraction) to pentadecanoic acid, was utilized for all radioactive fatty acids which were degraded. In all fractions except the stearic acid isolated from the unsaturated hydrogenated material the radioactive acids were diluted with twice their weight of non-radioactive acids.

(a) **Palmitophenone.**—A mixture of 823 mg. (3.2 mmoles) of palmitic acid, 1.5 ml. (20 mmoles) of pure thionyl chloride and 10 μl. of pyridine was heated, with stirring, to 40°, kept at this temperature for 30 minutes and the excess thionyl chloride removed under reduced pressure. The almost colorless palmitoyl chloride was dissolved in 3.5 ml. (30 mmoles) of dry benzene, cooled to 0° and 750 mg. (5.6 mmoles) of anhydrous aluminum chloride added in one portion. The stirring was continued for 12–16 hours at room temperature, dilute hydrochloric acid added and the organic layer diluted with hexane. Unreacted palmitic acid was removed by extraction with 5 ml. of 1 *N* sodium hydroxide; most of the sodium palmitate appeared as suspended particles in the aqueous layer and could be removed with the aqueous layer. (With pentadecanoic and lower acids, no sodium salt precipitated.) The organic layer was shaken with a mixture of 1:2 methanol–water to remove the last traces of sodium palmitate. After removal of the solvent from the hexane solution, the white crystalline palmitophenone was recrystallized twice from small quantities of hexane, m.p. 58–59° (lit.[26] 59–60°), yield 908 mg. (89%).

(b) **Pentadecanoic Acid.**—To a solution of 908 mg. (2.87 mmoles) of palmitophenone in 10 ml. of purified dioxane and 0.6 ml. of concentrated hydrochloric acid at 50°, there was added (through a Hershberg dropping funnel[27]) a solution of 0.49 ml. (3.9 mmoles) of freshly distilled isoamyl nitrite in 5 ml. of purified dioxane in the course of 45 minutes. The mixture was stirred with a magnetic stirrer during this reaction. After an additional 15 minutes at 50°, the solution was made basic by the addition of 12 ml. (36 mmoles) of 3 *N* sodium hydroxide. The yellow-brown solution was cooled to room temperature and 2.0 g. (10 mmoles) of *p*-toluenesulfonyl chloride was added in several portions to the stirred solution over a period of 20 minutes. The solution was again warmed to 50° and stirred at this temperature for two hours.

The solution was diluted with 300 ml. of water and the organic layer taken up in pentane. The separated aqueous layer contained a small amount of sodium pentadecanoate in the form of finely suspended particles but they could be dissolved by prolonged heating on a steam-bath. Slow cooling of the aqueous layer yielded larger particles and these were collected and yielded 30 mg. of pentadecanoic acid. After cooling the aqueous filtrate, hydrochloric acid was added and the solution extracted with several small portions of ether. The ethereal solution was evaporated to dryness in a sublimation apparatus and the residue sublimed at about 80° and 200 mm. The first few per cent. of sublimate was discarded and the sublimed benzoic acid, m.p.

(22) All combustions were performed by the Microanalytical Laboratory, College of Chemistry, University of California, Berkeley.

(23) E. Twitchell, *Ind. Eng. Chem.*, **13**, 806 (1921).

(24) Obtained from the Standard Oil Co. of Indiana.

(25) The "Eureka White Oil" was not separated directly from the isobutanol–water mixture since isobutyl alcohol is somewhat soluble in the oil and thus appreciable quantities of the salts of fatty acids can be dissolved in the isobutyl alcohol–oil mixture.

(26) R. Majima, K. Nagaoka and K. Yamada, *Ber.*, **55**, 215 (1922).

(27) E. B. Hershberg, "Organic Syntheses," Coll. Vol. II, J. Wiley and Sons, Inc., New York, N. Y., 1944, p. 129.

121–122°, weighed 278 mg. (79% based upon palmitophenone; 71% based upon palmitic acid).

The pentane solution was evaporated and the residual pentadecanonitrile was hydrolyzed by refluxing for 24 hours with 10 ml. of 15% potassium hydroxide in *n*-propanol. Dilution of the solution with hexane yielded a copious precipitate of sodium pentadecanoate. After removal of the salt by filtration, the filtrate was concentrated to dryness and triturated with hexane to remove neutral organic material. The remaining residue was combined with the soap obtained by filtration and the mixture acidified, extracted with pentane and the solvent evaporated. The crude acid was recrystallized twice from small volumes of pentane. The combined yield of pentadecanoic acid was 485 mg. (70% based upon ketone and 63% based upon palmitic acid), m.p. 51–52° (lit.[28] 52.3°).

Table I lists the results of all degradations. The melting points of all acids[28] and phenyl ketones[26] agreed with the published data. The yields were generally lower for the smaller-sized degradations.

TABLE I

DEGRADATION OF FATTY ACIDS

Fatty acids degraded		Yield of phenyl	Cleavage of phenyl ketone Yields (based on ketone)		
Name	Wt. used, mg.	ketone %	Wt. used, mg.	Benzoic acid, %	Fatty acid, %
Palmitic acid, saturated fraction					
Palmitic	823	89	908	79	70
Pentadecanoic	485	66	402	80	70
Myristic	211	79	211	82	41
Tridecanoic	64	90	74	49	23
Stearic acid, saturated fraction					
Stearic	478	90	521	82	65
Margaric	276	73	239	80	65
Palmitic	119	69	101	77	47
Pentadecanoic	37	60	28	Products lost	
Palmitic acid, unsaturated fraction					
Palmitic	259	81	260	72	51
Pentadecanoic	101	83	105	80	68
Myristic	54	62	42	72	52
Stearic acid, unsaturated fraction					
Stearic	869	90	943	60	59
Margaric	437	79	424	73	59
Palmitic	195	71	170	78	57
Pentadecanoic	74	74	68	Lost	37

(28) K. S. Markley, "Fatty Acids, their Chemistry and Physical Properties," Interscience Publishers, Inc., New York, N. Y., 1947, p. 114.

Radioactivity Determinations.—All samples were converted to barium carbonate by a wet oxidation procedure[29] and counted in the usual manner[30] on aluminum discs of 26 mm. diameter using a "Q-Gas" counter.[31] Self-absorption corrections were taken from the table given by Calvin, *et al.*,[32] Sufficient counts were taken to give a probable error of 5–8%. Table II lists the results of all activity determinations.

TABLE II

DISTRIBUTION OF ISOTOPE IN FATTY ACIDS

Fatty acid or benzoic acid	Specific activity[a]	Total activity[b]	Relative activity[c]
Saturated acids			
Palmitic, C_1–C_{16}	71	1140	100
Benzoic, C_1	22	154	13
Benzoic, C_2	2	14	1
Benzoic, C_3	20	140	12
Benzoic, C_4	3	21	2
Lauric, C_5–C_{16}	62	740	66
Stearic, C_1–C_{18}	57	1025	100
Benzoic, C_1	42	294	29
Benzoic, C_2	1	7	0.5
Palmitic, C_3–C_{18}	48	770	75
Benzoic, C_3	12	84	8
Benzoic, C_4	2	14	1
Unsaturated acids after hydrogenation			
Palmitic, C_1–C_{16}	32	510	100
Benzoic, C_1	11	77	15
Benzoic, C_2	1	7	1
Benzoic, C_3	10	70	14
Tridecanoic, C_4–C_{16}	30	390	76
Stearic, C_1–C_{18}	12	216	100
Benzoic, C_1	7	49	23
Benzoic, C_2	0.5	4	2
Benzoic, C_3	3	21	10
Myristic, C_5–C_{18}	10	140	67
Acetic, C_{17}–C_{18}	4	8	4

[a] Cts./min./mg. $BaCO_3$. [b] Specific activity × number of carbon atoms. [c] Total activity of fragment divided by total activity of fatty acid, expressed in per cent.

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(29) C. Entenman, S. R. Lerner, I. L. Chaikoff and W. G. Dauben, *Proc. Soc. Exper. Biol. Med.*, **70**, 364 (1949).

(30) W. G. Dauben, J. C. Reid and P. E. Yankwich, *Anal. Chem.*, **19**, 828 (1947).

(31) Obtained from the Nuclear Instrument Co., Chicago, Illinois.

(32) M. Calvin, C. Heidelberger, J. C. Reid, B. M. Tolbert and P. E. Yankwich, "Isotopic Carbon," John Wiley and Sons, Inc., New York, N. Y., 1949, p. 318.