tics of certain portions of the molecules. The $P\bar{1}$ space structure of these crystals and the orthorhombic substructure of the B′ configuration as well as the triclinic substructure of the A′ configuration were confirmed. Some strong infrared bands of the highly irregular triclinic A′ structure did not seem to be appreciably polarized. The data on group-frequency bands which did show clear-cut polarization were in agreement with prediction. The spectra of form A′, B′, and C′ crystals did not coincide with data obtained on KBr pellets. It is concluded that in these pellets fatty acids exist in a form which is not easy to duplicate under more conventional conditions.

REFERENCES

1. Coleman, J. E., and Swern, Daniel, J. Am. Oil Chemists' Soc., *35*, 675 (1958).
2. Hibben, J. H., Chem. Rev. *18*, 1 (1936).
3. Mann, J., and Thompson, H. W., Proc. Roy. Soc. (London), *A192*, 489 (1948).
4. Malkin, T., "Progress in the Chemistry of Fats," Vol. 1, Pergamon Press, 1957.
5. Newman, R., and Halford, R. S., J. Chem. Phys., *18*, 1276 (1950).
6. Susi, H., J. Am. Chem. Soc., *81*, 1535 (1959).
7. Susi, H., Spectrochim. Acta, *12*, 1063 (1959).
8. von Sydow, E., Arkiv Kemi, *9*, 231 (1956).
9. von Sydow, E., Acta Chem. Scand., *9*, 1119 (1955).
10. Unpublished work.

[Received February 8, 1960]

Hydrogenation of Methyl Oleate in Solvents [1]

E. R. COUSINS and R. O. FEUGE, Southern Regional Research Laboratory,[2] New Orleans, Louisiana

The hydrogenation of the oleic acid group was investigated with the objective of determining the effect of solvents on the reaction rate and the formation of positional and geometrical isomers. Methyl oleate, either alone or dissolved in one of several solvents, hexane, ethanol, *n*-butyl ether, or acetic acid, was hydrogenated to an iodine value of about 50 under atmospheric pressure and at 30°C. with palladium-on-carbon and the W-5 form of Raney nickel as catalysts.

Hydrogenation with palladium catalyst, with or without solvents, produced 76.6 to 79.1% *trans* bonds, based on the total double bonds. This is significantly greater than the 67% obtained previously. Hydrogenation products obtained with Raney nickel and solvents contained as little as 20.7% *trans* bonds at an iodine value of about 50. In two cases the *trans* bonds were equal to about one-third the positional isomers.

Positional isomers formed extensively when the Raney nickel was used in the absence of solvents and when the palladium catalyst was used. When the Raney nickel and solvents were used large proportions of double bonds were found in the original 9-position.

NUMEROUS investigators have concluded that the liquid-phase hydrogenation of unsaturated fatty acids and their esters in the presence of heterogeneous catalysts is greatly influenced by the solvent which is employed. Use of a solvent conceivably produces one or more of several effects. Among these are: a) a change in the viscosity of the liquid phase, which would affect mass transfer resistance, b) a change in the solubility of hydrogen in the liquid phase, c) a change in the adsorption characteristics of reactants on the catalyst surface, and d) dilution of the product to be hydrogenated.

Fokin (11), who apparently was among the first to present experimental data in this area, stated that the best solvents for the hydrogenation of oleic and other unsaturated acids in the presence of platinum black were water-soluble acids, alcohols, ether, and related compounds. Petroleum distillates, aromatic hydrocarbons, and higher alcohols were claimed to be less suitable. The use of solvents in the hydrogenation of oils has been claimed by Sanders (16) to result in increased selectivity without a concomitant increase in the formation of iso-oleic acid groups. According to him, the preferred solvents in order of preference were ethanol, methanol, isopropyl alcohol, cyclohexanol, acetone, and ethyl ether. Unpurified commercial hexane, petroleum ether and dioxane were deemed undesirable because they slowed the reaction rate. Vandenheuvel (19) found that the order of reaction during the hydrogenation of methyl oleate was influenced by the type of catalyst and the nature of the solvent employed. Sokol'skii *et al.* (17) investigated the influence of the nature of the solvent on the kinetics of the hydrogenation of cottonseed oil and concluded that the physicochemical properties of a solvent determine the temperature at which the maximum rate of hydrogenation occurs. Kaufmann (13) found that the hydrogenation of an oil in hexane at 35°C. produced very small amounts of iso-oleic acid groups.

Some of the conclusions cited above are not general facts and are valid only under the experimental conditions which were employed. Therefore data also have been obtained which on first examination appear contradictory. For example, in an investigation of the hydrogenation of methyl linoleate (7), the end-products obtained with palladium at 30°C. were found to be unaffected by the presence or absence of ethanol; the percentages of the residual double bonds in the various positions and the proportions of *trans* isomers were practically identical. Albright (2) found, on hydrogenating cottonseed oil alone and when dissolved in hexane, isopropyl alcohol, and isopropyl ether, that the rates of hydrogenation in solvent were less than the rates for the oil alone. Selectivity and the proportions of *trans* isomers produced were essentially unchanged by the presence of solvent. Albright's hydrogenations were carried out with a commercially-used nickel catalyst, at a temperature above 100°C., and at constant partial pressures of hydrogen. Thus much remains to be learned about hydrogenations in solvents.

The objective of the current investigation was to develop data on the formation of positional and geometrical isomers during the hydrogenation of methyl oleate in various solvents. Apparently no such investigation concerning the oleic acid group has heretofore been made.

[1] Presented at the 51st Annual Meeting of the American Oil Chemists' Society, Dallas, Tex., April 4–6, 1960.

[2] One of the laboratories of the Southern Utilization Research and Development Division, Agricultural Research Service, U. S. Department of Agriculture.

Experimental

Materials. The preparation of the methyl oleate used was described in detail in an earlier publication (8). To summarize the process briefly, methyl esters were prepared from a commercial pecan oil by methanolysis and the methyl oleate was separated from the mixed esters by a preliminary distillation, followed by crystallization from acetone and by a second distillation. The final product had an iodine value of 83.0 (theoretical, 85.6), a linoleate content of 0.13%, and a *trans* isomer content of 0.0%. Propyl gallate (0.01%) was added as an antioxidant.

The nickel catalyst was of the Raney type prepared essentially according to the method of Adkins and Billica (1). This method of preparation yields the so-called W-5 catalyst, which possesses a very high activity. Just prior to carrying out hydrogenations in solvents other than ethanol or in the absence of a solvent, the ethanol under which the catalyst was stored was removed by filtration and the appropriate solvent or methyl oleate was substituted.

The palladium catalyst, obtained from Baker and Co. Inc., was of the carbon-supported type and contained 10% by weight of the metal.

The solvents, all commercial products, were distilled in the presence of a sizable amount of a nickel catalyst to remove traces of any catalyst poisons which might have been present. In addition, the *n*-butyl ether was passed through a column of aluminum oxide to remove any peroxides.

Hydrogenation Apparatus and Procedure. The equipment and procedure were similar to those described previously (8) except for two modifications: the reaction vessel was immersed in a constant-temperature water bath, and a condenser was placed in the hydrogen outlet line to trap vaporized solvent and return it to the reaction vessel.

In the hydrogenations the charge was either 12.5 g. of methyl oleate dissolved in an equal weight of the selected solvent or 25.0 g. of methyl oleate alone. The reactions were carried out at 30°C. (±1°), atmospheric pressure, and a hydrogen flow rate of approximately 425 ml./min. Hydrogen was dispersed throughout the charge with the aid of a small, flat, fritted-glass disk. In some instances the rate of dispersion was so great that foaming resulted, and the flow of hydrogen had to be interrupted briefly to avoid loss of sample. All of the reactions were stopped at an iodine value of about 50.

When the proper amount of hydrogen had been consumed, the sample was taken from the system and the catalyst was removed by filtration. Then the sample was freed of solvent and stored under hydrogen in a refrigerator until analyzed.

Methods of Analysis. The techniques employed to determine the positions of the double bonds were essentially those described previously (5). They can be summarized as follows: Two grams of each sample were saponified with alcoholic potassium hydroxide, the alcohol was evaporated, the soaps were acidulated, and the resulting fatty acids were extracted. A 1-g. portion of the fatty acids was ozonized in ethyl acetate solution at −5°C., then treated with hydrogen peroxide. After removing the solvent and any short-chain monobasic acids, the residual mono- and dibasic acids were analyzed on two types of chromatographic columns. One column consisted of silicic acid coated with a citrate buffer (12), and the other of silicic acid coated with a glycine buffer (6).

Trans isomers were determined by a modification (9) of the infrared spectrophotometric method of Swern *et al.* (18).

Results and Discussion

Reaction Rates. The reaction order of each of the hydrogenations represented in Table I was apparently zero. The decrease in iodine value was directly proportional to the reaction time. The rate-determining step responsible for the zero order undoubtedly was not the same in all of the reactions though in most it probably was the rate of solution of hydrogen in the liquid phase.

The relative activity of the two types of catalysts cannot, of course, be compared directly on the basis of the data in Table I. Raney nickel is a nonsupported catalyst, and some of the nickel must function as a support, which means that the surface area per unit of weight is not exceptionally high. Palladium-on-carbon would be expected to possess more surface area per unit of weight of metal. Palladium is generally regarded as being the most active of hydrogenation catalysts. Recently Zajcew (20) presented data showing that decreasing the percentage of palladium from 0.02% to 0.0005% merely doubled the time required to hydrogenate cottonseed oil at 185°C., atmospheric pressure, and a fixed rate of hydrogen dispersion. In Runs 8–10 (Table I) the percentage of palladium probably could have been halved without affecting the hydrogenation rate.

The Raney nickel which was employed was much more active than are the nickel catalysts used in the commercial hydrogenation of oils. The latter catalysts do not appear to hydrogenate methyl oleate at room temperature and atmospheric pressure even when used in very high concentrations. The activity of Raney nickel of the W-5 modification decreases rapidly during storage. This may be one of the reasons that the Raney nickel used in Run 7 was not as active as that used in Run 2. Raney nickel did not hydrogenate methyl oleate in acetic acid solution, possibly because the acetic acid reacted with a portion of the catalyst.

In the hydrogenations, use of a solvent greatly increased the reaction rate. Increases ranged from approximately three- to eighteen-fold. Others also have found that the use of these and similar solvents increased the hydrogenation rate of fatty acid esters at low temperatures. The rate differences found among the solvents are not readily explained though some of the factors influencing the differences can be mentioned.

Because all hydrogenations were carried out at atmospheric pressure and 30°C., the partial pressure of hydrogen in the gas dispersed in the reacting systems did not remain constant. At 30°C. the vapor pressures of the pure solvents, measured in millimeters of mercury, are hexane, 188.6; ethanol, 78.8; *n*-butyl ether, approximately 9; and acetic acid, 20.6. The solubility of hydrogen in the solvent methyl oleate solutions and the viscosity of the solutions are other properties which could not be kept constant. However the most important variation probably was the manner in which the different solvents influenced adsorption of the reactants on the catalyst surfaces.

Geometrical Isomers. Hydrogenation of the oleic

TABLE I

Operational and Analytical Data on the Hydrogenation of Methyl Oleate in Solvents at 30°C.

Run No.	Hydrogenation Conditions			Hydrog. time, min.	Iodine value	*Trans* isomers, wt.%	*Trans* bonds,[a] %	Dicarboxylic acids obtained on oxidation, mol. %								
	Catalyst		Solvent													
	Conc., %	Type						C_6	C_7	C_8	C_9	C_{10}	C_{11}	C_{12}	C_{13}	C_{14}
1	3.4	Ni	None	200	47.5	42.5	76.6	4.6	16.7	24.1	17.0	12.6	7.4	7.4	5.6	4.6
2	3.4	Ni	Ethanol	34	49.2	20.1	35.0	5.7	9.1	16.1	45.3	7.9	6.3	4.1	2.8	2.7
3[b]	3.4	Ni	Hexane	33	50.9	12.3	20.7	11.6	16.0	14.6	44.7	7.8	1.9	0.0	1.5	1.9
4[b]	3.4	Ni	n-Butyl ether	11	51.0	17.6	29.5	2.8	9.2	16.0	54.5	9.2	4.7	1.7	0.9	1.1
5	3.4	Ni[c]	Hexane[d]	37	49.4	13.1	22.7	14.9	16.7	13.5	29.5	6.4	1.8	1.1	6.0	10.0
6[e]	3.4	Ni[f]	Ethanol	65	32.7	13.3	34.8	0.0	16.6	16.1	54.4	9.6	3.8	0.0	0.0	0.0
7[e]	3.4	Ni[f]	Ethanol	45	52.1	13.9	22.8	0.0	10.7	17.3	62.8	9.3	.....	.....	.....	.....
8	0.2	Pd	None	83	50.7	46.4	78.3	13.7	15.1	14.9	12.3	15.1	9.9	4.9	4.5	9.7
9	0.2	Pd	Ethanol	15	48.7	45.0	79.1	11.7	19.6	19.3	15.2	12.7	8.9	4.1	5.4	3.2
10	0.2	Pd	Acetic acid	31	54.0	49.4	78.3	8.4	14.1	15.4	15.1	14.7	11.7	8.7	6.7	5.2
Original methyl oleate					83.0	0.0	0.0	0.0	1.7	7.8	90.6	0.0	.....	.....	.....	.....

[a] Based on total number of *cis* and *trans* bonds. [b] Flow of hydrogen interrupted several times to permit foam to break. Reaction time does not include interruptions. [c] New preparation of catalyst used. [d] Citric acid in the amount of 0.1% suspended in hexane. [e] Data obtained in an earlier investigation (8). [f] Catalyst not of same preparations used in Runs 1–5.

acid group at 150° to 200°C. in the presence of nickel and palladium catalysts quickly isomerizes the residual double bonds to a *cis-trans* ratio of 1:2 (3,8,10). This ratio is theoretically attainable at equilibrium if the probabilities of all transitions are equal (4). To attain this equilibrium the *cis* and *trans* double bonds at similar positions along the carbon chain must be adsorbed on the catalyst with equal ease and must be hydrogenated with equal ease.

Among the hydrogenations represented in Table I, those carried out with the palladium catalyst (Runs 8–10) and that carried out with Raney nickel in the absence of a solvent (Run 1) did not attain this equilibrium, which corresponds to 67% *trans*. Instead the percentages of *trans* bonds ranged from 76.6 to 79.1. They differ from 67% by amounts greater than can be attributed to experimental error.

Two possible explanations can be advanced for the observed deviations, either the *trans* bonds are formed preferentially under the experimental conditions employed, or the *cis* bonds are hydrogenated (destroyed) preferentially. Support exists for the latter possibility. Patrikeev *et al.* (14) found that, on hydrogenating over Raney nickel a solution of maleic and fumaric acids in 96% ethanol, the maleic acid (*cis* bonds) hydrogenated at a more rapid rate. While this explanation might serve to explain the high percentages of *trans* found in Runs 1 and 8 through 10, it would fail to explain the proportions found in the other runs. Hence no conclusion can be drawn at this time.

In Runs 2 through 5 the percentages of *trans* isomers are far below 67%. A partial explanation might be that in each case a large proportion of the double bonds did not isomerize and instead remained at the 9-position in their original *cis* configuration. However this does not explain the fact that in Runs 3 and 5 the amounts of *trans* bonds were equal to only 37 and 32%, respectively, of the double bonds which migrated. Heretofore it had been concluded on the basis of experimental evidence that whenever new positional isomers were formed, the *cis-trans* ratio of the double bonds in the new positions was 1:2 (3,10). Present evidence indicates that under certain conditions of hydrogenation the ratio of *cis* to *trans* bonds in new positional isomers is greater than 1:2. Under these conditions either the *trans* bonds are hydrogenated preferentially or the *cis* bonds are formed preferentially.

In Run 5 citric acid was added to determine whether or not an acidic substance can change the course of hydrogenation. The citric acid did not dissolve completely, and its presence apparently had little if any effect. The differences observed on comparing Runs 3 and 5 might easily be attributed to differences in the two batches of catalyst.

On comparing Runs 2, 3, and 4 some evidence is found that the percentage of *trans* isomers increases as the polarity of the solvent increases. On comparing Runs 2 and 7, an indication is found that as the activity of the Raney nickel increases, the tendency to form *trans* bonds increases.

Positional Isomers. If the hydrogenation temperature is lowered while the other operating variables are kept constant, the proportion of positional isomers produced generally decreases (7,8). In an earlier investigation (8) the hydrogenation of methyl oleate was carried out under conditions like those represented in Run 1 except that a nickel catalyst prepared by electrolytic precipitation and dry reduction was used and the reaction was carried out at about 90° C., the lowest temperature at which this catalyst hydrogenated at an acceptable rate. Under these conditions 74.5% of the residual double bonds were found to be in the 9-position. These facts might imply that a more active nickel catalyst used at a still lower temperature would produce even fewer positional isomers. However the data for Run 1 in Table I do not bear this out. Apparently, increasing the activity of the nickel also increased its ability to produce positional isomers; in fact the W-5 form of Raney nickel used at 30°C. was about equivalent to the electrolytic nickel used at 200°C.

When the W-5 form of Raney nickel was used in the presence of solvents (Runs 2 through 5), the proportion of residual double bonds in the 9-position increased markedly. The reaction rate also increased. Addition of a solvent apparently produced the same effect as increasing the pressure in hydrogenations conducted in the absence of solvents.

The less active Raney nickel (Run 7) yielded a product having a higher percentage of double bonds in the 9-position than did the more-active Raney nickel (Run 2).

The palladium catalyst produced extensive migrations of the double bonds, and the presence or absence of solvent had no significant effect. Apparently the concentration of this highly reactive catalyst was such as to produce highly selective conditions, which are conducive to increasing the extent of migration of double bonds.

Concerning the direction of migration of the double bonds, some hydrogenated products (Runs 3, 5, 6, and

9) were found to have more double bonds below than above the 9-position. This does not necessarily mean that the predominant direction of migration was toward the ester linkage. As the distance of a double bond from the ester linkage increases, its rate of hydrogenation increases (15). Hence a sizable proportion of double bonds which migrated to the outer positions may have been preferentially hydrogenated and did not appear in the end product.

Acknowledgments

The authors wish to acknowledge the assistance of Robert T. O'Connor, Elizabeth R. McCall, and Donold Mitcham, who performed the spectrophotometric analyses.

REFERENCES

1. Adkins, H., and Billica, H. R., J. Am. Chem. Soc., *70*, 695–698 (1948).
2. Albright, L. F., Wei, C. H., and Woods, J. M., J. Am. Oil Chemists' Soc., *37*, 315–320 (1960).
3. Allen, R. R., and Kiess, A. A., J. Am. Oil Chemists' Soc., *32*, 400–405 (1955).
4. Blekkingh, J. J. A., Bull. soc. chim., France, *1950*, 278–282.
5. Chahine, M. H., Cousins, E. R., and Feuge, R. O., J. Am. Oil Chemists' Soc., *35*, 396–401 (1958).
6. Corcoran, G. B., Anal. Chem., *28*, 168–171 (1956).
7. Cousins, E. R., Guice, W. A., and Feuge, R. O., J. Am. Oil Chemists' Soc., *36*, 24–28 (1959).
8. Feuge, R. O., and Cousins, E. R., J. Am. Oil Chemists' Soc., *37*, 267–271 (1960).
9. Feuge, R. O., Cousins, E. R., Fore, S. P., DuPré, E. F., and O'Connor, R. T., J. Am. Oil Chemists' Soc., *30*, 454–460 (1953).
10. Feuge, R. O., Pepper, M. B. Jr., O'Connor, R. T., and Field, E. T., J. Am. Oil Chemists' Soc., *28*, 420–426 (1951).
11. Fokin, S., J. Russ. Phys.-Chem. Soc., *40*, 276–321 (1908).
12. Higuchi, T., Hill, N. C., and Corcoran, G. B., Anal. Chem., *24*, 491–493 (1952).
13. Kaufmann, H. P., U. S. 2,852,541 (1958).
14. Patrikeev, V. V., Balandin, A. A., and Khidekel, M. L., Bull. Acad. Sci., U.S.S.R., Div. Chem. Sci. S.S.R., *1958*, 395–401 (English translation).
15. Pigulevskii, G. V., and Antamonov, P. A., J. Gen. Chem. (U.S.S.R.), *12*, 510–517 (in English, 517) (1942).
16. Sanders, J. H. (Procter and Gamble Co.), U. S. 2,520,440 (1950).
17. Sokol'skii, D. V., Melekhina, L. S., and Perunova, L. I., J. Appl. Chem. U.S.S.R., *30*, 1869–1875 (1957) (English translation).
18. Swern, Daniel, Knight, H. B., Shreve, O. D., and Heether, M. R., J. Am. Oil Chemists' Soc., *27*, 17–21 (1950).
19. Vandenheuvel, F. A., J. Am. Oil Chemists' Soc., *33*, 347–350 (1956).
20. Zajcew, M., J. Am. Oil Chemists' Soc., *37*, 11–14 (1960).

[Received April 15, 1960]

Biogenesis of Polyunsaturated Acid in Fish[1]

JAMES F. MEAD,[2] MITSU KAYAMA,[3] and RAYMOND REISER,[4] Laboratory of Nuclear Medicine and Radiation Biology, Department of Biophysics and Nuclear Medicine, and Department of Physiological Chemistry, School of Medicine, University of California, Los Angeles

IT HAS BEEN KNOWN for many years that the fatty acids of fish are more highly unsaturated and have greater average chain-lengths than those of most mammals. That the differences may be even more basic became apparent as the structures of the fatty acids were determined. Despite their high unsaturation fish oils, in general, did not serve as a source of essential fatty acids (1). This would indicate that they do not belong to the linoleic family

$$[CH_3(CH_2)_4 - CH = \text{termination}]$$

(2,3) as do most of the mammalian polyunsaturated acids. Structure determinations of individual fatty acids have confirmed these ideas. For example, Klenk and his coworkers (4,5) have shown that most of the polyunsaturated fatty acids of herring oil belong to the linolenic family ($CH_3 - CH_2 - CH =$ termination) (2,3), a finding which appears to agree with those of Stoffel, Insull, and Ahrens (6) for menhaden oil.

If the fatty acids of fish are so different from those of mammals, the question of their derivation and transformations is of interest. It has been shown in these laboratories (3) that the synthesis of polyunsaturated fatty acids in the rat (and presumably other mammals) is accomplished by the addition of double bonds in the divinyl methane relationship to unsaturated fatty acids derived from the diet or synthesized in the animal body. In mammals the parent acid is usually linoleic from the diet, thus leading to the family of essential fatty acids including γ-linolenic, 8,11,14-eicosatrienoic, and arachidonic acids. In the absence of linoleic acid, dietary linolenic and biosynthetic oleic and palmitoleic acids can also be converted to higher polyunsaturated acids. The problems to be considered in this connection are whether the polyunsaturated acids of the fish are predominantly of the linolenic family because the fish, unlike the mammal, can synthesize their precursor or whether they are formed by the familiar process from dietary fatty acids largely of the linolenic type. The possibility that fish possess, to a greater degree than mammals, the ability to extend oleic acid must also be considered.

Klenk (personal communication) found that, following the injections of acetic-1-C^{14} acid into fish, ozonolytic degradation of their polyunsaturated acids acids revealed some activity in the malonic acid fraction. This could only have arisen by a synthetic process, but the nature of the fatty acids degraded was uncertain. Reiser and his coworkers (7,8) found that the nature of the fish fatty acids is markedly influenced by diet and came to the conclusion that although the differences between the fatty acid compositions of marine- and fresh-water fish are due to differences in their diets, they also have a marked ability to synthesize polyunsaturated but not necessarily essential fatty acids. When these authors placed fish on a fat-free diet, their polyunsaturated fatty acids were reduced but they did not appear to develop any deficiency symptoms. This would be expected from the results of similar treatment of adult rats.

In the present experiment an attempt was made to ascertain which, if any, polyunsaturated acids might be synthesized by fish and to study further the effect of dietary fat on their deposited fatty acids.

Experimental

Treatment of Animals. Three mature female *Tilapia mossambica* which had been raised in salt water and

[1] This paper is based on work performed under contract No. AT-04-1-gen-12 between the Atomic Energy Commission and the University of California at Los Angeles.
[2] Part of this work was carried out at the Hawaii Marine Laboratory, where it was supported by a grant from the Pauley Fund.
[3] Visiting scientist from Tohoku University, Sendai, Japan. Partially supported by a training grant from the National Heart Institute, Bethesda, Md. Present address: Department of Biochemistry and Nutrition, Texas Agricultural Experiment Station, College Station, Tex.
[4] Department of Biochemistry and Nutrition, Texas Agricultural Experiment Station, College Station, Tex.