THE COURSE OF HYDROGENATION OF SARDINE OIL

MAROMI TAKEDA and YOSHIYUKI TOYAMA

Department of Applied Chemistry

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Many detailed studies on the course of hydrogenation of vegetable oils and their component fatty acids have hitherto been reported. But the course of hydrogenation of marine animal oils has not been so closely studied as that of vegetable oils, and there is apparently lack of conformity in previous studies. According to Ubbelohde and Svanoe,1) clupanodonic acid is converted to linoleic (diethenoid) acid without forming linolenic (triethenoid) acid in the course of hydrogenation of marine animal oils. Kita and his co-workers2) also came to the same conclusion in their studies on the hydrogenation of herring oil. Armstrong and Hilditch3) found that polyethenoid acids are first preferentially hydrogenated in the hydrogenation of whale oil as is the case with cotton seed and linseed oils. Ueno4) also reported that in the hydrogenation of sardine oil the highly unsaturated acids and unsaturated acids of smaller carbon number are more readily hydrogenated than the less unsaturated acids and unsaturated acids of larger carbon number. Ueno and Okamura5) studied on the effect of the condition of hydrogenation of plaice oil on the formation of solid unsaturated acids. According to Richardson *et al.*,6) highly unsaturated acids are preferentially hydrogenated to diethenoid or monoethenoid acids in the initial stage of hydrogenation of whale and menhaden oils, and when the iodine value of oil has fallen to 84, almost all of the highly unsaturated acids have disappeared. Toyama and Tsuchiya7) found that in the hydrogenation of a highly unsaturated methyl ester fraction prepared from sardine oil, solid acids are not formed in considerable amounts so long as the highly unsaturated acids giving ether-insoluble bromide have not disappeared, but in the case of the hydrogenation with platinum black at ordinary temperature the tendency of such selective hydrogenation is not brought out markedly. Takano *et al.* studied on the hydrogenation of sardine,8) herring9) and plaice10) oils with the results that highly unsaturated acids are first hydrogenated resulting in an accumulation of so-called "iso-oleic" and liquid monoethenoid acids which reach their respective maxima when the highly unsaturated acids giving petroleum ether-insoluble bromide have disappeared and then decrease parallel to each other. In the hydrogenation of the methyl esters of monoethenoid acids prepared from sardine oil,11) lower esters were more rapidly hydrogenated than higher esters. Fractionation of the fatty acids obtained from a partially hydrogenated sardine oil12) showed that diethenoid and triethenoid acids were rich in higher boiling fraction while saturated acids were rich in lower boiling fraction. Furthermore, Takano13) 14) 15) separated a triethenoid acid fraction from a partially hydrogenated product of methyl clupanodonate, and indicated the presence of three isomerides.

While previous studies, cited above, on the course of hydrogenation of marine animal oils and their component fatty acids appear to have chief resort to the bromination test and the determination of solid acids, the authors examined the course of hydrogenation of sardine oil with resort to the ultraviolet absorption measurement of the fatty acids and their alkali-isomerized product and the infrared absorption measurement in addition to the bromination of fatty acids and the determination of saturated acids.

Experimental

1. Hydrogenation of sardine oil. The sardine oil used for hydrogenation was prepared from a crude sardine oil produced in the vicinity of Hakodate in the spring season, 1954, by refining with alkali followed by bleaching with activated clay in this laboratory. It had n_D^{20} 1.4794, acid value 0.5, saponification value 192.3 and iodine value (Wijs) 168.3.

Hydrogenation was conducted at about 180°C by blowing hydrogen through the oil (80 g) to which was added 2% of nickel catalyst (flake catalyst supplied by the Nikki Kagaku Co.). Eight samples were taken out at suitable time intervals. It required eight hours to take out the last sample. This is presumably due to the lack of sufficient agitation by blowing alone. Refractive index of hydrogenated oils and the melting point and iodine value of their fatty acids are shown in Table 1.

TABLE 1. Refractive Index of Hydrogenated Oils and Characteristics of Their Fatty Acids

Hydrogenated oil sample No.	Appearance at room temperatnre	n_D^{60}	Fatty acids	
			m.p. (°C)	Iodine value
Before hydrogenation	Deposits some solid	1.4642	—	176.4
1	Almost nonfluid	1.4618	31.5–32	139.8
2	Soft solid	1.4608	31 –32	127.1
3	″	1.4587	31 –31.5	114.5
4	″	1.4569	31 –31.5	102.0
5	″	1.4549	32 –32.5	89.4
6	″	1.4535	33 –34	77.7
7	Hard solid	1.4502	40.5–42	52.3
8	″	1.4494	42.5–43.5	46.7

2. Determination of saturated acids and bromination test. Instead of determining the saturated acids in the total fatty acids of each sample in Table 1, the saturated methyl esters in the methyl esters of total fatty acids were determined by the permanganate-acetone oxidation method. A minor difference between the percentage of the saturated esters in the methyl esters of total fatty acids and the percentage of the saturated acids in the total fatty acids was neglected, and the figures obtained for the esters were designated as the figures for the fatty acids in Table 2.

Bromination test was conducted in the following way: Each sample of the fatty acids shown in Table 1 was dissolved in about twenty times of ether. A little excess of bromine was added dropwise to the solution under cooling below 5°C. After the solution was allowed to stand for two hours, the ether-insoluble bromide formed was filtered, washed three times with ether using 20 cc each time, and dried at a temperature below 60°C. The ether filtrate and washings were united and freed from bromine, and the ether was distilled off. Thirty cc of hexane was added to the residue, and the hexane-insoluble bromide consisting of white solid and resinous matter was separated from the solution, washed two times with hexane using 30 cc each time and finally vacuum dried at a temperature below 60°C. The results are shown in Table 2.

TABLE 2. Determination of Saturated Acids and Bromination Test

Fatty acids sample No.	Saturated acids (%)	Iodine v. of unsaturated acids*	Ether-insoluble bromide (%)	Hexane-insoluble bromide (%)
Before hydrogenation	26.9	241.3	52.1	46.7
1	28.9	196.6	25.2	39.9
2	30.3	182.4	15.8	28.3
3	32.0	168.4	5.6	18.4
4	34.2	155.0	2.3	8.8
5	36.5	140.8	0.7	3.1
6	40.7	131.0	—	—
7	54.3	114.4	—	—
8	58.6	112.8	—	—

Notes: * Calculated from the iodine value of fatty acids (Table 1) and the content of saturated acids.

3. Formation of conjugated acids and *trans*-unsaturated acids in the course of hydrogenation. The ultraviolet absorption for the fatty acids of each sample was measured in ethanol. The content of conjugated acids in the fatty acids was

TABLE 3. Determination of Conjugated Acids by Ultraviolet Absorption Measurement

Fatty acids sample No.	Conjugated acids (%)	
	Dienoic	Trienoic
Before hydrogenation	3.0	—
1	4.5	0.7
2	5.7	0.4
3	4.1	0.2
4	2.1	—
5	0.7	—
6	0.3	—
7	—	—
8	—	—

TABLE 4. Infrared Absorption Spectrum of Fatty Acids

Fatty acids sample No.	Method	Wave length position of observed absorption band (μ)	Vibrating group giving rise to observed absorption band
Before hydrogenation	Methyl ester, without using solvent ..	—	—
1	Methyl ester, KBr-tablet method	10.33; 10.09	Isolated *trans*; *cis, trans, trans*-conjugated
2	〃	10.33; 10.09	Isolated *trans*; *cis, trans, trans*-conjugated
3	〃	10.33	Isolated *trans*
4	Fatty acids in CS_2	10.33	Isolated *trans*
5	〃	〃	〃
6	〃	〃	〃
7	〃	〃	〃
8	〃	〃	〃

Notes: Samples Nos. 4-8 showed the absorption band at 10.69 μ due to carboxyl group.

calculated from the ultraviolet absorption data on an assumption that the conjugated dienoic and trienoic acids in the sample under examination have the same spectral characteristics with conjugated C_{18}-dienoic and C_{18}-trienoic acids.[16] Infrared absorption spectrum of the fatty acids in the neighbourhood of 10μ was measured in order to examine the presence of any *trans*-unsaturated acids. The results are shown in Tables 3 and 4.

4. Estimation of polyethenoid acids by ultraviolet absorption measurement of alkali-isomerized fatty acids. Each sample of the fatty acids was isomerized under the condition of 21% KOH-glycol, 180°C and 15 minutes with a current of nitrogen, and the specific extinction coefficients of the alkali-isomerized product at 233, 268, 316, 347 and 374 mμ were measured (Table 5). The contents of diethenoid, triethenoid, tetraethenoid, pentaethenoid and hexaetheonid acids were calculated by applying the formula given by Hammond and Lundberg[17] on an assumption that each polyethenoid acid member, diethenoid to hexaethenoid, in the sample under examination is same with the corresponding member in the formula of Hammond and Lundberg in its behavior in alkali-isomerization and the spectral characteristics of its alkali-isomerized product. The results are shown in Tables 6 and 7.

TABLE 5. Ultraviolet Absorption Measurement of Alkali-isomerized Fatty Acids

Fatty acids sample No.	Specific extinction coefficient				
	233 mμ	268 mμ	316 mμ	347 mμ	374 mμ
Before hydrogenation	19.45	18.71	16.88	9.69	3.20
1	18.38	11.46^b	9.21	3.91^d	0.79
2	16.25	10.50	7.14^c	3.46	0.65
3	11.56	6.08	3.34	1.35	0.25
4	8.01^a	2.96	1.27^c	0.31	—
5	3.80	1.19	0.32	0.15^d	—
6	2.14	—	—	—	—
7	1.06	—	—	—	—
8	0.91	—	—	—	—

Notes: The figures marked *a*, *b*, *c* and *d* are the specific extinction coefficients at 234, 269, 315 and 346 mμ, respectively. The absortion maxima were observed at these wave lengths instead of 233, 268, 316 and 347 mμ for other samples.

TABLE 6. Composition of the Unsaturated Acids (Pentaethenoid Acids Are Assumed to Be C_{22})

Fatty acids sample No.	Ethenoid acids (%)						Calcd. iodine value of the total fatty acids
	Mono-(C_{18})	Di-(C_{18})	Tri-(C_{18})	Tetra-(C_{20})	Penta-(C_{22})	Hexa (C_{22})	
Before hydrogenation	31.6	4.4	2.7	10.2	13.3	10.9	180.9
1	40.2	10.2	3.7	8.0	6.3	2.7	129.3
2	42.5	9.3	4.6	5.4	5.7	2.2	119.1
3	50.1	8.1	3.7	3.0	2.2	0.9	93.6
4	54.9	6.7	2.1	1.5	0.6	—	75.2
5	58.5	3.4	1.0	0.3	0.3	—	64.0
6	57.0	2.3	—	—	—	—	55.4
7	44.6	1.1	—	—	—	—	42.1
8	40.4	1.0	—	—	—	—	38.1

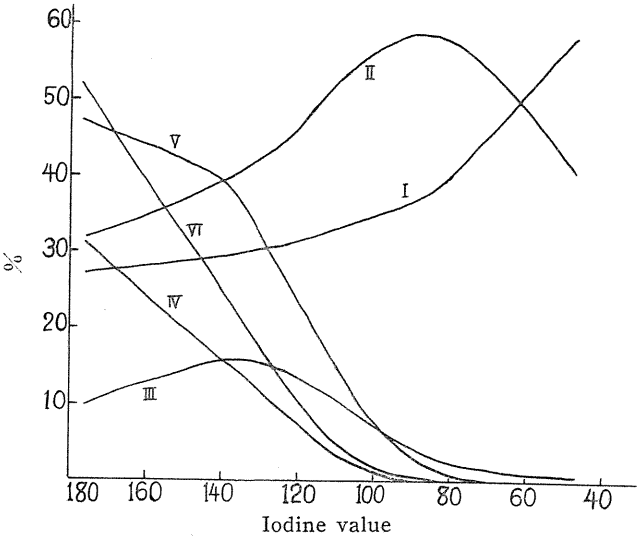
Notes: Monoethenoid acids % = 100 − (saturated acids % + polyethenoid acids %). Iodine value of the total fatty acids was calculated from the composition given in this table.

TABLE 7. Composition of the Unsaturated Acids (Pentaethenoid Acids Are Assumed to Be C_{20})

Fatty acids sample No.	Ethenoid acids (%)						Calcd. iodine value of the total fatty acids
	Mono- (C_{18})	Di-(C_{18})	Tri- (C_{18})	Tetra- (C_{20})	Penta- (C_{20})	Hexa- (C_{22})	
Before hydrogenation	32.4	5.5	4.4	12.2	7.7	10.9	176.0
1	40.2	11.0	4.6	9.0	3.6	2.7	127.6
2	42.6	9.8	5.5	6.3	3.3	2.2	117.8
3	50.4	8.2	3.8	3.4	1.3	0.9	92.7
4	55.1	6.7	2.0	1.6	0.4	—	74.7
5	58.6	3.4	1.0	0.3	0.2	—	63.7
6	57.0	2.3	—	—	—	—	55.4
7	44.6	1.1	—	—	—	—	42.1
8	40.4	1.0	—	—	—	—	38.1

Discussion

Based on the data in Tables 1, 2, 6 and 7, the relationship between the iodine value of fatty acids, the yield of ether-insoluble bromide and hexane-insoluble bromide, and the amounts of saturated, monoethenoid, diethenoid and triethenoid, and more highly unsaturated acids are graphically shown in Figure 1. As seen from Figure 1, the ether-insoluble bromide and hexane-insoluble bromide decrease rapidly with the progress of hydrogenation, and completely disappear in the stage of hydrogenation when the iodine value of fatty acids falls to a value lying between 89 (sample No. 5) and 78 (sample No. 6). The rate of increase of saturated acids is



I. Saturated acids
II. Monoethenoid acids
III. Diethenoid and triethenoid acids
IV. Tetraethenoid, pentaethenoid and hexaethenoid acids
V. Hexane-insoluble bromide
VI. Ether-insoluble bromide

FIG. 1. Relationship between the iodine value of fatty acids, the yields of ether-insoluble bromide and hexane-insoluble bromide, and the amounts of various fatty acid components.

small in the initial stage of hydrogenation, becomes larger with the progress of hydrogenation and tends to increase very rapidly when the ether-insoluble bromide and hexane-insoluble bromide disappear.

The compositions of unsaturated acids in Tables 6 and 7 were calculated by assuming monoethenoid, diethenoid and triethenoid as C_{18}, tetraethenoid as C_{20}, pentaethenoid as C_{22} or C_{20} and hexaethenoid as C_{22}. But it is quite doubtful whether this assumption may reasonably be permitted. Especially the assumption that monoethenoid, diethenoid and triethenoid acids are all C_{18} appears rather unreasonable. Comparing the calculated iodine values in Table 6 and 7 and the observed iodine values in Table 1, the observed iodine values for the fatty acids of hydrogenated oil samples are always somewhat higher than the calculated iodine values, while the observed iodine value for the fatty acids of sardine oil before hydrogenation is a little lower than the calculated iodine value. The main reason for this inconsistency is considered to be the formation of diethenoid or other polyethenoid acids, which are difficultly isomerized with alkali, in the course of hydrogenation of sardine oil, since it has been known that "iso-linoleic" acid[18)19)20)] which is difficultly isomerized with alkali is formed in the course of hydrogenation of vegetable oils containing linolenic acid. Consequently, the contents of polyethenoid acids shown in Tables 6 and 7 appear to be a little smaller than the real contents on the whole. Although it is seen from these considerations that the data for unsaturated acids in Tables 6 and 7 are not altogether correct, an inspection of these data reveals some significant features pertaining to the transitions of respective polyethenoid acids in the course of hydrogenation. Hexaethenoid, pentaethenoid and tetraethenoid acids decrease with the progress of hydrogenation. There is no accumulation of pentaethenoid and tetraethenoid acids in the course of hydrogenation. Diethenoid acids show a small accumulation in the early stage of hydrogenation, reach a maximum and then decrease. Also triethenoid acids show a minor accumulation in the relatively early stage of hydrogenation, reach a maximum and then decrease.

Almost all of the polyethenoid acids except only about 2% of diethenoid acids disappear between the fatty acid samples, No. 5 (I.V. 89) and No. 6 (I.V. 78). This is quite consistent with the results of bromination test. On the other hand, however, it should be mentioned that since the fatty acid samples succeeding No. 5 and No. 6 have calculated iodine values which are lower than observed values, the unsaturated acids designated as monoethenoid acids in Tables 6 and 7 are likely to contain some polyethenoid acids, chiefly diethenoid acids, which are difficultly isomerized with alkali and give no hexane-insoluble bromide. While Takano[14)] obtained a partially hydrogenated product of methyl clupanodonate which gives 0.14 % of ether-insoluble bromide and 142.5% of petroleum ether-insoluble bromide, the results of the present study on the hydrogenation of sardine oil and a previous study by Toyama and Tsuchiya[7)] on the hydrogenation of a highly unsaturated methyl ester fraction prepared from sardine oil indicated that when the fatty acids giving ether-insoluble bromide disappear, the fatty acids giving petroleum ether-insoluble or hexane-insoluble bromide are present only in a minor amount, and so the fatty acids which contain a small proportion of the polyehtenoid acids giving ether-insoluble bromide and a large proportion of the polyethenoid acids giving hexane-insoluble bromide are not formed during hydrogenation.

Monoethenoid acids gradually increase with the progress of hydrogenation, reach a maximum in the stage of hydrogenation when the iodine value of fatty acids lies between 89 and 78, and then decrease. Namely, monoethenoid acids reach a maximum when polyethenoid acids almost disappear.

As shown in Table 3, about 3% of conjugated dienoic acids are present in the fatty acids of sardine oil before hydrogenation. The conjugated dienoic acids are also produced during hydrogenation, and show a small accumulation in the early stage of hydrogenation, but they gradually decrease with a further progress of hydrogenation until their complete disappearance. Also the conjugated trienoic acids are formed and show an accumulation, though very small, in the early stage of hydrogenation. As it is known that *trans*-unsaturated acids are formed in the course of hydrogenation of fatty oils, the presence of isolated *trans*-configuration in all of the samples, Nos. 1–8, were verified by the infrared absorption measurement. Two samples, No. 1 and No. 2, were found to contain also *cis-trans-trans* conjugated configuration. Although the presence of *cis-trans* (absorption maxima at 10.18 μ and 10.55 μ) and *trans-trans* (absorption maximum at 10.12 μ) conjugated configurations in some samples is highly probable, the absorption maxima corresponding to these configurations could not be observed presumably due to a minor content of these configurations.

Summary

Sardine oil (iodine value of its fatty acids; 176) was hydrogenated, and samples hydrogenated to different degrees were prepared. The course of hydrogenation was investigated by estimating the amount of saturated acids and the yields of ether-insoluble bromide and hexane-insoluble bromide for the fatty acids of each sample, and also by the application of ultraviolet absorption measurement of the fatty acids and their alkali-isomerized product and the infrared absorption measurement of the fatty acids. The results are summarized as follows.

1. The yields of ether-insoluble bromide and hexane-insoluble bromide from the fatty acids decrease with the progress of hydrogenation, and when the iodine value of fatty acids falls to a range of 89–78, neither ether-insoluble bromide nor hexane-insoluble bromide are formed. The rate of increase of saturated acids is small in the initial stage of hydrogenation, but it becomes remarkably large when the fatty acids giving ether-insoluble bromide disappear.

2. The composition of polyethenoid acids in each sample was calculated from the ultraviolet absorption data for the alkali-isomerized product of fatty acids on an assumption that each polyethenoid acid member, diethenoid to hexaethenoid, in the sample under examination is same with the corresponding member in the formula of Hammond and Lundberg in its behavior in alkali-isomerization and the spectral characteristics of its alkali-isomerized product. There was no accumulation of pentaethenoid and tetraethenoid acids in the course of hydrogenation. Diethenoid acids showed a small accumulation in the early stage of hydrogenation. Also triethenoid acids showed a minor accumulation in the relatively early stage of hydrogenation. When the iodine value of fatty acids has fallen to 89–78, almost all of the polyethenoid acids except about 2% of diethenoid acids disappeared. Monoethenoid acids increased with the progress of hydrogenation, reached a maximum when the iodine value of fatty acids was lowered to 89–78, and then decreased. It should, however, be mentioned that some polyethenoid acids, chiefly diethenoid acids, which are difficultly isomerized by alkali, are presumably formed in the

course of hydrogenation, and consequently the amount of polyethenoid acids estimated by the ultraviolet absorption measurement appears to be a little smaller than the real amount on the whole.

3. Conjugated dienoic and trienoic acids were formed in the course of hydrogenation. Both acids showed a minor accumulation, exceedingly small in the case of trienoic acids, in the early stage of hydrogenation and disappeared in the latter stage of hydrogenation. The presence of isolated *trans*-configuration in all of the hydrogenated samples was verified by the infrared absorption measurement. Also *cis-trans-trans* configuration was present in some samples in the early stage of hydrogenation.

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