How Is Chemical Interesterification Initiated: Nucleophilic Substitution or α-Proton Abstraction?

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ABSTRACT: Esters of carboxylic acids including 2-methylhexanoic, 2-methylbutyric, 2,2-dimethyl-4-pentenoic, palmitic, and oleic acids were tested as substrates in methoxide-catalyzed interesterification and transesterification. The aliphatic acid esters participated in the ester–ester interchange upon addition of catalytic sodium methoxide. Their isopropyl esters also produced methyl esters on heating with sodium methoxide. The esters of α-methyl-substituted acids did not participate in the ester–ester interchange. Their isopropyl esters did not react with methoxide to produce methyl esters. However, upon addition of methanol with sodium methoxide, their methyl esters were produced. These results indicate that the first step in interesterification is possibly that methoxide abstracts the α-hydrogen of an ester to form a carbanion. Interesterification is then likely completed *via* a Claisen condensation mechanism involving the β-keto ester anion as the active intermediate. The β-keto ester anion contains positively charged ketone and acyl carbons that are active sites for nucleophilic attack by anions such as methoxide and glycerinate, which would produce a methyl ester or rearrange acyls randomly. On the other hand, transesterification is a nucleophilic substitution by methoxide at the acyl carbon in the presence of methanol.

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KEY WORDS: Carbanion, Claisen condensation, decarboxylation, enolate, interesterification, β-keto ester anion, laurone, mechanism, palmitone, transesterification.

Chemical interesterification rearranges the distribution of FA in TG. This rearrangement changes the TG composition, and therefore the physical properties of the oils, and does not lead to the formation of *trans* FA.

Interesterification can occur without catalysts at very high temperatures, such as 250°C, or catalytically under milder conditions, such as 60°C. Many compounds have been patented as interesterification catalysts including metal salts, alkali hydroxide, alkoxide (alkylates), and alkali metals (1–3). Sodium methoxide is the most commonly used catalyst for chemical interesterification. The reaction is commonly divided into three periods: induction, interchange, and completion. During the induction period (ranging from seconds to minutes), sodium methoxide reacts with glycerides to produce an intermediate commonly referred to as the “real catalyst” (1); no interchange of acyl groups takes place during this period. Although the existence of this real catalyst is not doubted by those skilled in the art, experimental evidence revealing the identity or structure of the catalytic species is still lacking. Once the real catalyst is formed, the interchange period of the reaction begins. Acyl groups continue to interchange until thermodynamic equilibrium is reached and no further net change in the distribution of FA occurs. At this point, the reaction reaches the third period, completion, which is usually terminated out by quenching with water to destroy the real catalyst.

Two reaction mechanisms have been proposed for interesterification. Weiss *et al.* (4) suggested that interesterification was initiated when methoxide abstracted the α-hydrogen of an ester to form an enolate anion (α-carbanion ester) that then formed a β-keto ester *via* Claisen condensation (5). Interesterification was then understood to occur by repetitive nucleophilic substitution of β-keto esters by glyceride anions (diglycerinate) on the ketone carbon or acyl carbon until thermodynamic equilibrium was reached. This β-keto ester mechanism was supported by the detection of an IR absorption peak at 6.4 μm (1562.5 cm^{-1}) in the oil and water phases of sodium methoxide-catalyzed interesterification reactions (4). This evidence has been challenged because this absorption is also characteristic of the ionized carboxyl group of soap that is commonly formed as a by-product in sodium methoxide-catalyzed interesterification (1,6). Coenen (7) proposed that interesterification was initiated by a direct nucleophilic addition and substitution of methoxide on the acyl carbon of a glycerol ester to form a diglycerinate. Interesterification was achieved by repetitive nucleophilic substitution of diglycerinate moieties at the acyl carbons of TG. So far, no additional experimental research to support either mechanism has been published.

In this research, the reaction between sodium methoxide and a series of acid esters with and without α-methyl branching was studied to further elucidate the mechanism of chemical interesterification.

EXPERIMENTAL PROCEDURES

Chemicals. Sodium methoxide was purchased from Aldrich (Milwaukee, WI) as a 30% solution in methanol and the sodium methoxide powder was prepared by evaporating the methanol at about 90°C just prior to use. 2-Methylhexanoic acid (99% purity by GC), 2-methylbutyric acid (98%), 2,2-dimethyl-4-pentenoic acid (95%), palmitic acid (99%), and oleic acid (99%) were purchased from Sigma Chemical Co. (St. Louis, MO). The acids were converted into methyl and isopropyl esters by the following acid-catalyzed esterification.

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Acid-catalyzed esterification. Methods recommended by Christie (8) were modified as follows: 100 mg of the acid was heated with 6 mL of alcohol (methanol or isopropanol) containing 2% (vol/vol) concentrated sulfuric acid in a sealed vial at around 95°C for an hour. Then 5 mL of hexane and 3 mL of a 5% NaCl solution were added and mixed well. After settling, the hexane layer was separated and washed three times with 3 mL of 2% (wt/vol) potassium bicarbonate or until the aqueous layer tested neutral by pH paper. The hexane layer was dried over molecular sieves and analyzed for purity by GC. The synthesized esters were further purified by washing with alkali to remove residual acids. The purified esters were at least 99.7% pure as analyzed by GC.

GC (9). The esters were analyzed by a Hewlett-Packard (Wilmington, DE) model 5890 gas chromatograph fitted with an FID and a 15 m × 0.244 mm Alltech (Deerfield, IL) DB-23 capillary column. The column was run isothermally at 200°C for long-chain FA esters (oleate and palmitate) and at 90°C for the other short-chain esters. The peaks were identified using the pure standards prepared as above and integrated for quantification. The compositions were expressed as the percentage of the integrated peak areas.

Interesterification. An ester sample of about 30 mg was dissolved in 1 mL of hexane. Sodium methoxide powder was added while stirring at about 60°C, and the sample was incubated for 1 min in screw-capped vials. The vials were then centrifuged, and 2 µL of hexane supernatant was injected into a gas chromatograph for analysis. The amounts of esters used were chosen so that the final solutions could be analyzed using GC directly without further dilution or concentration. The amount of sodium methoxide used in this research was higher than that in a neat oil interesterification (0.3–0.5%) to ensure the initiation of reaction under the diluted hexane solution. The following reactions were conducted.

(*i*) *Interesterification of methyl palmitate and isopropyl oleate.* A 30-mg mixture of equal weights of methyl palmitate and isopropyl oleate were mixed in 1 mL hexane. Two interesterification reactions were conducted, using 1.5 and 3.6 mg of sodium methoxide, respectively.

(ii) Interesterification of methyl 2-methylbutyrate and isopropyl oleate. Methyl 2-methyl butyrate and isopropyl oleate (~1:2 w/w ratio) were mixed in 1 mL hexane, and 3.6 mg of sodium methoxide was added.

(iii) Interesterification of isopropyl oleate. Isopropyl oleate (30 mg) was dissolved in vials, each containing 1 mL of hexane; 3 and 6 mg of sodium methoxide, respectively, were added to the reaction vials.

(iv) Interesterification and transesterification (methanolysis) using isopropyl 2-methylhexanoate. Interesterification was conducted by reacting 30 mg of esters in 1 mL of hexane with 3, 6, 9, and 18 mg of sodium methoxide, respectively. Transesterification (methanolysis) was conducted by sequential additions of 5, 35, and 100 µL of methanol to the reaction mixture containing 18 mg sodium methoxide. The mixtures were heated for 1 min at 60°C with stirring after each addition of methanol and sampled prior to the next addition of methanol. The hexane layer was analyzed using GC.

(v) Interesterification and transesterification (methanolysis) of isopropyl 2,2-dimethyl-4-pentenoate. In interesterification, 30 mg of ester was dissolved in 1 mL of hexane and reacted with 9, 18, and 27 mg of sodium methoxide, respectively. Transesterification was conducted by sequential addition of 30, 60, 120, and 240 µL of methanol to the mixture containing 30 mg of ester and 27 mg of sodium methoxide.

RESULTS AND DISCUSSION

All reactions and analyses were conducted in duplicate. The difference between results was within 7%, and therefore only one run of each experiment is discussed.

Yield of acid-catalyzed esterification. Acid-catalyzed esterification produced acid esters with at least 98% purity for all acids except 2,2-dimethyl-4-pentenoic acid, which yielded about 86% ester. After alkali treatment, the residual acids were removed, and all the purified esters were at least 99.7% pure as analyzed by GC.

Interesterification of methyl palmitate and isopropyl oleate. The two reactants were mixed in about 1:1 weight ratio, as confirmed by GC (Table 1). After addition of sodium methoxide and heating for 1 min at 60°C, two new esters (isopropyl palmitate and methyl oleate) were formed. The amount of those newly formed esters increased when the dose of sodium methoxide increased from 1.5 to 3.6 g (0.79 to 5.36% for isopropyl palmitate and 1.88 to 14.53% for methyl oleate). More methyl oleate than isopropyl palmitate was produced; therefore, methoxide not only catalyzed the interesterification reaction but also participated as a reactant in the reaction. The results confirmed that the experimental conditions were adequate for interesterification.

Interesterification of methyl 2-methylbutyrate and isopropyl oleate. In this reaction, methyl 2-methylbutyrate was used to replace methyl palmitate in reaction with isopropyl oleate. Methyl 2-methylbutyrate contains a methyl branch at its α-position and therefore has only one α-hydrogen that is not acidic enough for methoxide to abstract (5). The experimental results listed in Table 2 show that there was no ester interchange or interesterification between methyl 2-methylbutyrate and isopropyl oleate because only methyl oleate was produced. The amount of methyl 2-methylbutyrate decreased to 23.16% after reaction from the initial 38.58% (39% decrease), and the amount of isopropyl oleate increased slightly from 61.42 to 62.06%, which might be caused by evaporation during heating as methyl 2-methylbutyrate has a low b.p. The trace amount (0.23%) of isopropyl ester might be formed *via*

TABLE 1
Interesterification of Methyl Palmitate and Isopropyl Oleate

$NaOCH_3$	Palmitate (%)		Oleate (%)	
added[a] (mg)	Methyl	Isopropyl	Methyl	Isopropyl
0.0	49.9	—[b]	—	50.1
1.5	50.89	0.79	1.88	46.43
3.6	45.74	5.36	14.53	34.37

[a] As catalyst.
[b] Not detected.

TABLE 2
Interesterification of Methyl 2-Methylbutyrate and Isopropyl Oleate

$NaOCH_3$	2-Methylbutyrate (%)		Oleate (%)	
added[a] (mg)	Methyl	Isopropyl	Methyl	Isopropyl
0	38.58	—[b]	—	61.42
3.6	23.16	0.23	13.63	62.06

[a] As catalyst.
[b] Not detected.

transesterification as described below. If interesterification were indeed initiated by nucleophilic substitution of methoxide at the acyl carbon, isopropyl 2-methylbutyrate should also be produced at a level similar to that of isopropyl palmitate in Table 1. It is very unlikely that the 2-isopropylbutyrate was formed and then lost by evaporation because its amount would still be higher than 0.23% at the loss rate of 39%, which was consistent with the results below from the isopropyl esters of 2-methylhexanoic and 2,2-dimethyl-4-pentenoic acids. Methyl 2-methylbutyrate has an α-methyl branch at its α-position and contains only one α-hydrogen. It is known that the basicity of sodium methoxide is not sufficient to abstract the only α-hydrogen of an α-substituted ester, and a strong base such as sodium triphenylmethide is needed (5). Isopropyl oleate contains an α-hydrogen that is acidic enough to be abstracted by methoxide, and therefore methyl oleate was produced. Although the isopropylate anion is large in size compared with methoxide, isopropyl esters have been successfully synthesized in high yield by the acid-catalyzed esterification as above including those α-substituted acids. Lee *et al.* (10) also reported the syntheses of isopropyl esters using the base-catalyzed transesterification. Therefore, failure to produce isopropyl 2-methylbutyrate is unlikely to be due to steric hindrance of the isopropylate anion and the α-methyl branch. This is the first experimental evidence showing the essential role of the active α-hydrogen for interesterification.

Interesterification of isopropyl oleate. This test was used to examine the reaction of an individual ester with sodium methoxide. On heating with sodium methoxide, isopropyl oleate reacted with methoxide and produced methyl oleate (Table 3). The amount of methyl oleate was doubled when the sodium methoxide was increased from 3 to 6 mg. This result shows that an aliphatic acid ester can react with methoxide to produce a methyl ester, although it is not clear if this is the result of nucleophilic substitution or α-proton abstraction.

TABLE 3
Interesterification of Isopropyl Oleate with Sodium Methoxide

$NaOCH_3$ added (mg)	Isopropyl ester (%)	Methyl ester (%)
0	99.59	—[a]
3	94.26	5.74
6	88.32	11.68

[a] Not detected.

TABLE 4
Interesterification of Isopropyl 2-Methylhexanoate with Sodium Methoxide

$NaOCH_3$		Isopropyl ester	Methyl ester	Isopropanol	Methanol
(mg)	(μmol)	(%)	(%)	(%)	(%)
0	0	98.04	0.00	0.11	0.00
3	0.06	99.76	0.20	0.02	0.02
6	0.11	99.48	0.44	0.02	0.06
9	0.17	98.91	0.94	0.06	0.09
18	0.33	98.01	1.44	0.13	0.42

Interesterification and transesterification (methanolysis) using isopropyl 2-methylhexanoate. 2-Methylhexanoate contains a methyl branch at the α-carbon of its acid moiety. With addition of up to 18 mg of sodium methoxide (0.33:0.17 molar ratio of methoxide to ester), only a trace amount of methyl ester (<1.44%) was formed (Table 4). Thus, an ester having no active α-hydrogen did not react with sodium methoxide to form methyl esters, indicating that methoxide was not able to attack the acyl carbon to carry out a nucleophilic substitution and produce methyl 2-methylhexanoate. Further analyses revealed that trace amounts of methanol existed in the reaction mixture (0.02–0.42%), which may account for the trace amounts of methyl ester produced through methanolysis as described below. Methanol can be formed *in situ* by sodium methoxide with the α-hydrogen of an ester or any compounds containing acidic hydrogen, or it may be brought in as a impurity along with sodium methoxide. Trace amounts of isopropanol were also formed as the product of methanolysis or transesterification.

To test the effect of methanol on the reaction, methanol was added to the interesterification mixture. Table 5 presents the results after up to 100 μL of methanol was added to the reaction mixture containing 18 mg of sodium methoxide. With addition of methanol, significant amounts of methyl ester (1.87–7.7%) were produced. The amount of isopropanol also increased in the mixture as the result of transesterification. Methanol is a polar solvent and can form hydrogen bonds with acyl oxygens, which may activate the acyl carbon of isopropyl ester so that the methoxide can attack nucleophilically at the acyl carbon and produce the methyl ester. The results also argue against the possibility that α-substitution can inhibit methoxide from making a nucleophilic attack at the acyl carbon of an acid ester by steric hindrance.

Interesterification and transesterification (methanolysis) of isopropyl 2,2-dimethyl-4-pentenoate. 2,2-Dimethyl-4-pentenoate has two methyl branches at its α-position and contains no α-hydrogen. Table 6 shows that results obtained with this double-substituted acid ester were similar to those obtained

TABLE 5
Transesterification (methanolysis) of Isopropyl 2-Methylhexanoate

Methanol (μL)	Isopropyl ester (%)	Methyl ester (%)	Isopropanol (%)	Methanol (%)
0[a]	98.01	1.44	0.13	0.42
5	96.35	1.87	0.21	1.56
35	94.36	3.65	0.31	1.68
100	86.46	7.7	0.67	5.17

[a] The mixture containing 18 mg of $NaOCH_3$ from Table 4.

TABLE 6
Interesterification and Methanolysis of Isopropyl 2,2-Dimethyl-4-pentenoate

	Isopropyl ester (%)	Methyl ester (%)
$NaOCH_3$ (mg)		
0	100	0
9	100	0
18	99.79	0.21
27	99.77	0.23
Methanol (μL)[a]		
30	98.9	1.1
60	98.29	1.71
120	98.07	1.93
240	97.34	2.66

[a] Methanol was added to the mixture above that contained 27 mg $NaOCH_3$.

with the single-substituted acid esters. Without added methanol, only a trace amount of methyl ester (<0.23%) was formed even within the presence of 27 mg of sodium methoxide in the reaction mixture. After methanol was added to the reaction mixture containing 27 mg of sodium methoxide, significant amounts (1.10–2.66%) of methyl ester were produced, albeit less than the amount of methyl ester formed with a single-substituted acid ester (Table 4).

The results presented in the foregoing paragraphs show that methoxide-catalyzed interesterification is very likely initiated by α-proton abstraction and that methoxide functions as an alkali. Transesterification (methanolysis) is a nucleophilic substitution reaction in the presence of methanol, and methoxide functions as a nucleophile.

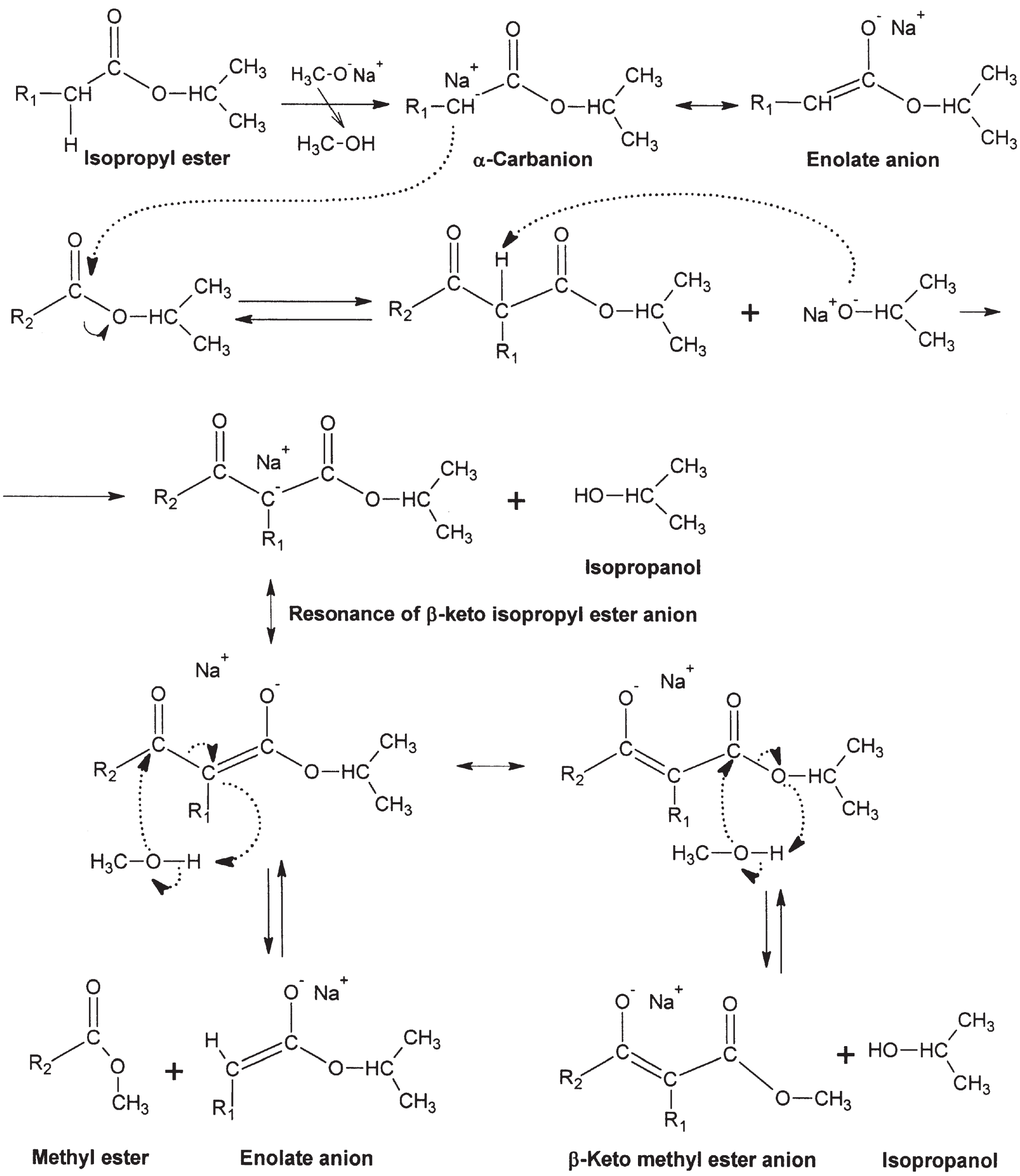

FIG. 1. Formation of methyl ester from isopropyl ester *via* the β-keto ester anion mechanism.

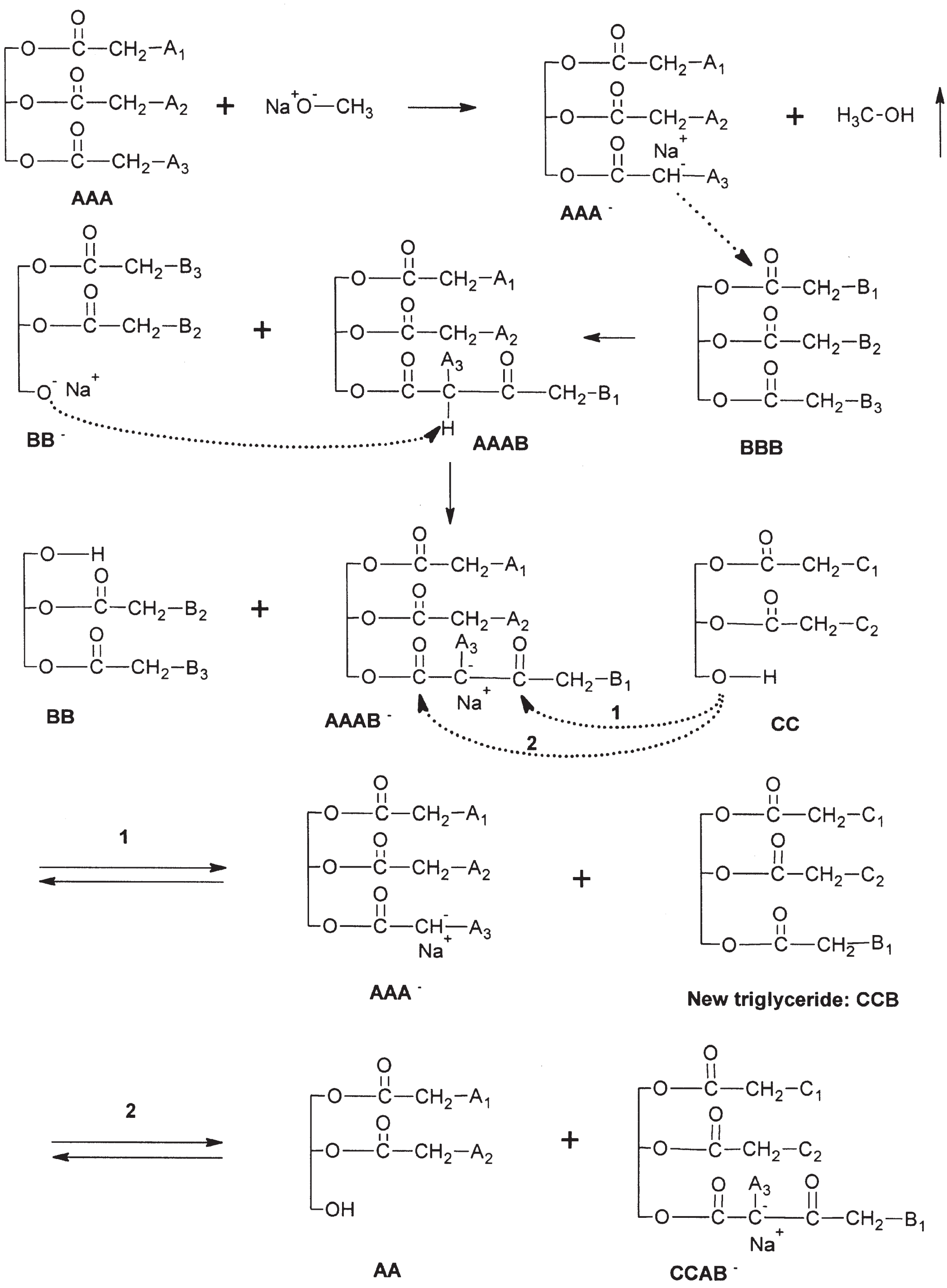

FIG. 2. The β-keto ester anion is the acyl donor for the formation of the new TG.

Figure 1 proposes a new interesterification scheme for the formation of methyl ester from isopropyl ester and sodium methoxide *via* the Claisen condensation mechanism. The first step is to abstract the α-hydrogen by methoxide and thus form an α-carbanion, a resonance isomer of an enolate anion that is a strong nucleophilic agent. A β-keto ester is formed when an α-carbanion is condensed with another isopropyl ester *via* Claisen condensation by nucleophilic substitution. A β-keto ester contains an acidic α-hydrogen ($pK_a = 9–13$) and therefore further reacts with sodium isopropylate to form a β-keto isopropyl ester anion and isopropanol. The β-keto isopropyl ester anion contains three negatively charged centers (two oxygens and one carbanion) that polarize the keto and acyl carbons into becoming more positively charged, which may activate those centers for a nucleophilic reaction by an anion or alcohol. When the methanol formed in the first step attacks at the acyl carbon of the β-keto isopropyl ester

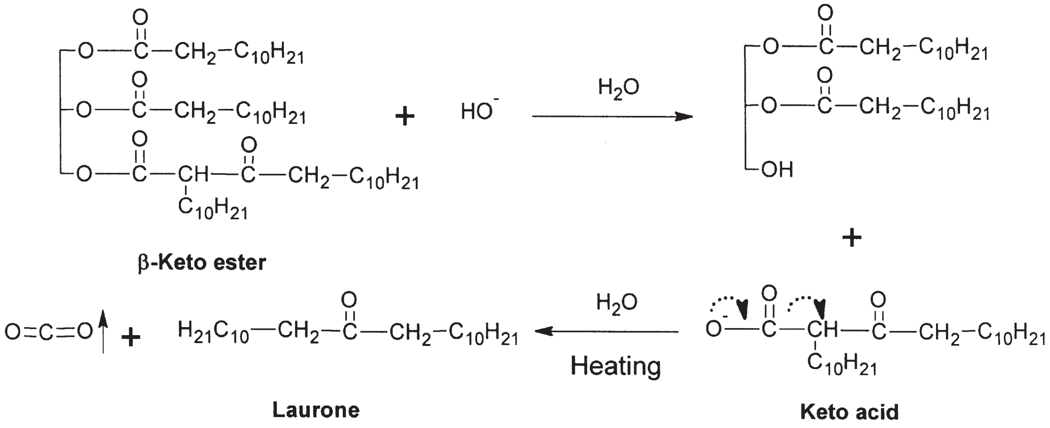

FIG. 3. Formation of laurone by the decarboxylation of β-keto acid.

anion, a β-keto methyl ester anion and isopropanol are formed, resulting in a net transesterification. When the methanol attacks at the keto carbon, a methyl ester and an enolate (α-carbanion) are formed, resulting in a net ester–ester exchange or interesterification. The β-keto ester anion contains a conjugated system that has three resonance isomers and would have an absorption in the near UV region; this accounts for the reddish-brown color that has been cited as an indicator of interesterification (1) and that is used for reaction control (11).

Figure 2 shows the suggested scheme for the rearrangement of FA in TG AAA, BBB, and CCC to form a new hybrid TG CCB. The first step is to abstract the α-hydrogen in AAA by methoxide to form AAA⁻ (α-carbon anion TG). AAA⁻ then reacts with triglyceride BBB *via* a Claisen condensation to form AAAB (β-keto ester TG) and BB⁻ (diglycerinate). The latter two then exchange a proton to form AAAB⁻ (β-keto ester TG anion) and DG BB. AAAB⁻ then reacts with another DG (CC) that is generated through the same mechanism as BB. When the nucleophilic substitution occurs at the keto group, a new hybrid TG (CCB) is formed and AAA⁻ is regenerated. When the reaction occurs at an acyl group, a new DG (AA) and a new hybrid β-keto ester TG anion (CCAB⁻) are produced. Repeated reactions will lead to randomization of FA among the TG.

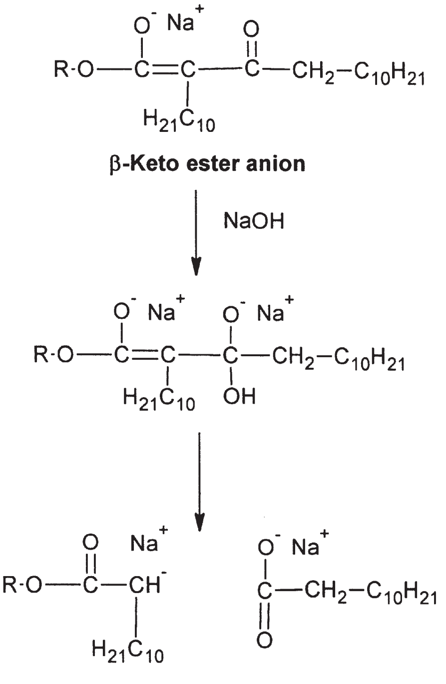

FIG. 4. Formation of soap during interesterification.

This β-keto ester anion mechanism can also explain other side reactions and by-products produced during interesterification. Huyghebaert *et al.* (12) isolated a series of long-chain ketones such as laurone ($C_{11}H_{23}COC_{11}H_{23}$) and palmitone ($C_{15}H_{31}COC_{15}H_{31}$) from three butter substitutes, and those ketones did not exist in natural butters. Figure 3 shows a proposed mechanism for the formation of laurone from the β-keto ester containing lauric acid. After interesterification is complete, water is usually added to ensure the reaction is stopped. Water quenches the β-keto ester anion into a β-keto ester that is then hydrolyzed into a β-keto acid. β-Keto acid is not stable and is easily decarboxylated to produce a ketone upon heating. A carbon is lost due to decarboxylation, which results in odd-chain (C_{11} or C_{15}) ketones from lauric and palmitic acids, respectively. Other long-chain ketones can be formed similarly. This decarboxylation is referred to as ketonic cleavage (13).

The β-keto ester can also undergo acid cleavage (13) to form soap (Fig. 4). Soap is a well-known by-product from interesterification and causes a 3–5% neutral oil loss in a typical interesterification. It is commonly believed that soap is formed after interesterification when water is added. According to this mechanism, soap can be formed from the β-keto ester anion with sodium hydroxide during interesterification. Sodium hydroxide can be introduced as an impurity in sodium methoxide and can be formed when sodium methoxide is exposed to moisture. The author has observed that soap is mainly formed during oil interesterification before water is added, and it can be separated by centrifugation. The formation of soap turns the oil from a clear reddish brown solution into a turbid reddish solution because soap is less soluble in oil. Similarly, methyl ester can be produced as another by-product when methanol is formed from methoxide. Ahibo-Coffy and Prome (14) reported that glycerol-β-keto ester synthesized using palmitic acid produced mono- and dipalmitoyl glycerol, palmitone, and mixed glycerides containing β-keto ester and palmitate upon heating with alkaline K_2CO_3. This is an additional support for the β-keto ester anion mechanism for interesterification.

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REFERENCES

1. Screenivasan, B., Interesterification of Fats, *J. Am. Oil Chem. Soc. 55*:796–805 (1978).
2. Rozenaal, A., Interesterification of Oils and Fats, *INFORM 3*:1232–1237 (1992).
3. Marangoni, A.G., and D. Rousseau, Engineering Triacylglycerols: The Role of Interesterification, *Trends Food Sci. Technol. 6*:329–335 (1995).
4. Weiss, T.J., G.A. Jacobson, and L.H. Wieldermann, Reaction Mechanics of Sodium Methoxide Treatment of Lard, *J. Am. Oil Chem. Soc. 38*:396–399 (1961).
5. Solomons, T.W.G., *Organic Chemistry*, 3rd edn., John Wiley & Sons, New York, 1984, pp. 897–902.
6. Bellamy, L.J., *Infrared Spectra of Complex Molecules*, John Wiley & Sons, New York, 1954, pp. 149–150.
7. Coenen, J.W.E., Fractionnement et Interesterification des Corps Gras dans la Perspective du Marche Mondial des Matières et des Produits Finis. II. Interesterification, *Rev. Fr. Corps Gras 21*:403–413 (1974).
8. Christie, W.W., *Lipid Analysis*, 2nd edn., Pergamon Press, Oxford, United Kingdom, 1982, pp. 52–53.
9. Liu, L., and E.G. Hammond, Phenylethyl Esters of Fatty Acids for the Analytical Resolution of Petroselineate and Oleate, *J. Am. Oil Chem. Soc.72*:749–751 (1995).
10. Lee, I., L.A. Johnson, and E.G. Hammond, Use of Branched-Chain Esters to Reduce the Crystallization Temperature of Biodiesel, *J. Am. Oil Chem. Soc. 72*:1155–1160 (1995).
11. Liu, L., and D. Lampert, Partial Interesterification of Triacylglycerols, U.S. Patent 6,238,926 (2001).
12. Huyghebaert, A., H. Hendrickx, N. Schamp, and L. DeBuyck, Occurrence of Ketones in Products Resembling Butter, *Fette Seifen Anstrichm. 72*: 289–293 (1970).
13. Beyer, H., and W. Walter, *Handbook of Organic Chemistry*, translated and amended from the 22nd German edn. by D. Lloyd, Prentice Hall, New York, 1996, pp. 293–298.
14. Ahibo-Coffy A., and J.-C. Prome, Transesterification de β-Cetoester par des Polyols: Intervention de Clivages Nucleophiles Intramoleculaires, *Tetrahedron Lett. 18*:1503–1506 (1976).

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