

## Identification of Novel Decenoic Acids in Heated Butter

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**Novel decenoic acids such as (*E*)-4-decenoic acid and (*E*)- and (*Z*)-5,6-decenoic acid were detected as minor components in heated butter using GC and GC/MS. The formation mechanism of these novel decenoic acids is discussed on the basis of the result of the reaction of  $\delta$ -decalactone with active clay in a model experiment.**

**Key words:** (*E*)-4-decenoic acid; (*E*)- and (*Z*)-5,6-decenoic acid; flavor; identification; heated butter

Butter is widely used as a seasoning in cooking, suggesting that it plays an important role in the formation of flavors during heating.<sup>1–4)</sup> A number of papers have reported the flavor constituents of butter.<sup>5–8)</sup> The fatty acid components of butter, in particular, have been studied for a considerable period of time, and the constituent compounds have been reported to be of importance in giving butter its flavor.<sup>9,10)</sup> However, concurrent studies on the amounts of these compounds in butter have revealed that the flavor was almost entirely due to *n*-alkanoic acids such as acetic acid, butyric acid, octanoic acid, decanoic acid, dodecanoic acid, and tetradecanoic acid.<sup>1–3,5,6)</sup> Butter was also observed to contain unsaturated alkanolic acids such as (*Z*)-9-octadecenoic acid (oleic acid) and (*Z*),(*Z*)-9,12-octadecadienoic acid (linoleic acid), but these do not contribute to butter flavor owing to their non-volatility.<sup>10,11)</sup> Among the series of decenoic acids that were synthesized, it was demonstrated that (*E*)-6-decenoic acid, in particular, had a highly significant intense milky flavor as compared to others.<sup>12,13)</sup> Our area of interest was in ascertaining the presence of these decenoic acids, including (*E*)-6-decenoic acid, in heated butter. 9-Decenoic acid has been isolated from butter,<sup>14–16)</sup> but limited information is available on the position, number, and geometry of the double bonds of other decenoic acids. In this paper, we describe the identification and formation mechanism of novel decenoic acids in heated butter.

## Materials and Methods

A commercial unsalted sweet butter was purchased from Yukijirushi Co. (Tokyo). Ethyl acetate, hexane, sodium hydroxide, sulfuric acid, sodium acetate, sodium

chloride, active clay, and trimethylsilyldiazomethane in hexane were obtained from reliable commercial sources and used without further purification. (*Z*)-4-Decenoic acid and methyl ester were purchased from Orils (Paris, France). (*E*)-4-Decenoic acid was purchased from Inoue Perfumery Co., Ltd. (Tokyo). [(*E*)- and (*Z*)]-5- and -6-Decenoic acids were prepared using a known pathway. 9-Decenoic acid was purchased from Tokyo Kasei Kogyo Co., Ltd. (Tokyo). The methyl esters of (*E*)-4-decenoic acid, [(*E*)- and (*Z*)]-5- and -6-decenoic acid, and 9-decenoic acid were obtained from the reaction of the corresponding acids and trimethylsilyldiazomethane in hexane, benzene, and methanol at 25 °C for 30 min.  $\gamma$ - and  $\delta$ -Decalactone were production of Soda Aromatic Co., Ltd. (Tokyo).

**Analysis of acidic fraction of heated butter.** Butter (98.0 g) was placed in a three neck round bottom flask equipped with a thermometer, a mechanical stirring system, and a Liebig condenser for removal of water. The flask was heated to 170 °C with stirring for 5 h and then cooled to 25 °C. Ethyl acetate (200 g) and 200 g of 2.5 wt % sodium hydroxide solution were added and extracted. The water layer was extracted with 100 g of ethyl acetate. The water layer was then washed with 10 wt % sulfuric acid solution, and 100 g of ethyl acetate was added to the mixture. The upper organic layer was washed with 100 g of 5 wt % sodium acetate solution and 100 g of 20 wt % sodium chloride solution. It was subsequently evaporated to give 82.3 mg of a brown-colored syrup. This syrup was analyzed by GC and GC/MS. An Agilent 6890 GC system (Agilent Technologies, Palo Alto CA) equipped with a TC-WAX (30 m  $\times$  0.25 mm i.d.; film thickness of 0.25  $\mu$ m; GL Sciences, Tokyo) and flame ionization detector (FID) or an Agilent 5973 mass selective detector (MSD) was used for the analysis. The operating conditions were as follows: injector temperature, 250 °C; detector temperature, 250 °C; ionization voltage, 70 eV; ion source temperature, 150 °C; helium carrier gas flow, constant pressure mode of 14.99 psi (FID) or 2.56 psi (MSD); oven temperature program, from 65 °C to 120 °C at a rate of 3 °C/min and then from 120 °C to 250 °C at a rate of 5 °C/min. The sample (0.2  $\mu$ l) was injected in a split ratio of 1:150.

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**Analysis of methylated acidic fractions of heated butter.** The foregoing 11.9 mg of syrup in 1 ml of methanol–benzene (2:8) was added to 0.3 ml of trimethylsilyldiazomethane–hexane (1:9) and the mixture was allowed to stand for 30 min at 25 °C. Then it was evaporated to give 13.4 mg of oil. The methylated oil was analyzed by GC and GC/MS. An Agilent 6890 GC equipped with an HP-88 (100 m × 0.25 mm i.d.; film thickness of 0.25 µm; Agilent Technologies, Palo Alto CA) and an FID or Agilent 5973 MSD was used for the analysis. The operating conditions were as follows: injector temperature, 250 °C; detector temperature, 280 °C; ionization voltage, 70 eV; ion source temperature, 150 °C; helium carrier gas flow, 3.0 ml/min; and oven temperature program, 155 °C (constant temperature). The sample (0.2 µl) was injected in a split ratio of 1:50.

**Analysis of the reaction of  $\delta$ -decalactone with active clay.**  $\delta$ -Decalactone (100 g) and 2 g of active clay was placed in a three neck round bottom flask equipped with a thermometer and a mechanical stirring system. The flask was heated to 170 °C with stirring for 5 h and then cooled to 25 °C. The reaction mixture was filtered using a filter paper to afford 94.9 g of oil. It was chromatographed on a silica gel using ethyl acetate–hexane (1:10) as an eluent to yield 18.5 mg of oil. The oil thus obtained was analyzed by GC and MS. The analysis conditions were similar to those maintained during the analysis of the acidic fraction of heated butter described above.

## Results and Discussion

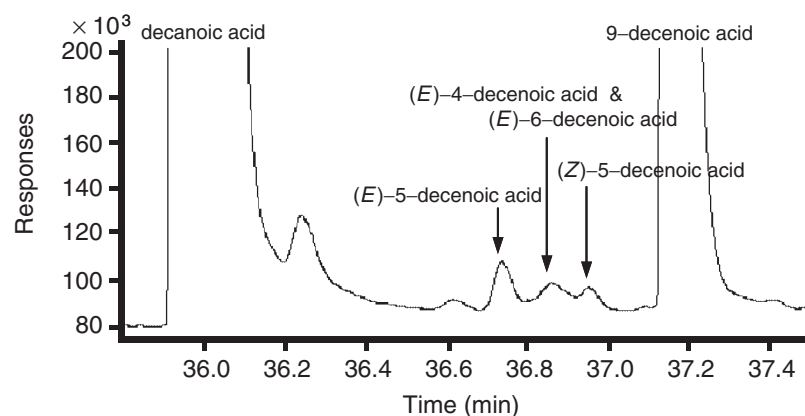
The relative GC peak area (%) of each chemical obtained from fatty acid fractions of heated butter is listed in Table 1. C-2, -4, -6, -8, -10, -12, -14, -16, -18, and -18(9) *n*-alkanoic acids were identified as the major components. The existence of these fatty acids has been reported previously.<sup>10,15,16</sup> The GC analysis under consideration, having retention times between 35.85 and 37.45 min, is also indicated in Fig. 1.

The retention times of GC and MS for (*E*)-5-, (*Z*)-5-, and 9-decenoic acids were identical to those of the above described authentic samples. The retention times and relative GC peak areas of (*E*)-5-, (*Z*)-5-, and 9-decenoic acids were 36.73, 36.95, and 37.13 min and 0.023%, 0.010%, and 0.946%, respectively. Our attempts to identify (*E*)-4- and (*E*)-6-decenoic acids using several GC conditions, however, were unsuccessful because their retention times and MS patterns were identical. The GC peak of the (*E*)-4- and/or (*E*)-6-decenoic acid(s) appeared at 36.85 min. We were unable to identify (*Z*)-6-decenoic acid either since its GC peak was very close to that of 9-decenoic acid, which was a large peak.

(*E*)-4-, (*E*)-6-, and (*Z*)-6-Decenoic acids were identified by transforming their acids into corresponding methyl esters using trimethylsilyldiazomethane. The GC data considered in this analysis shows retention times between 6.20 and 6.75 min, obtained from the methyl

**Table 1.** Acid Fractions Identified in Heated Butter

Compound	Retention time (min)	GC peak area (%)
acetic acid	13.30	2.53
butyric acid	19.99	1.32
hexanoic acid	26.46	3.85
octanoic acid	31.56	3.03
$\gamma$ -decalactone	33.27	0.012
$\delta$ -decalactone	34.31	0.226
decanoic acid	35.94	8.86
( <i>E</i> )-5-decenoic acid	36.73	0.023
( <i>E</i> )-4-decenoic acid and (or) ( <i>E</i> )-6-decenoic acid	36.85	0.019
( <i>Z</i> )-5-decenoic acid	36.95	0.010
9-decenoic acid	37.13	0.946
$\delta$ -dodecalactone	38.79	0.207
dodecanoic acid	39.85	5.88
tetradecanoic acid	43.46	8.82
hexadecanoic acid	47.28	27.6
stearic acid	52.33	8.56
oleic acid	53.33	15.83
linoleic acid	55.04	0.582



**Fig. 1.** GC Analysis (35.85–37.45 min) of the Fatty Acid Fractions of Heated Butter.

ester of fatty acid fraction of heated butter, as shown in Fig. 2.

The retention times and relative GC peak areas of methyl (*E*)-4- and -(*E*)-6-decenoate were 6.39 and 6.51 min and 0.023% and 0.011% respectively. The retention time of methyl (*Z*)-6-decenoate was 6.71 min. Since the GC peak did not completely separate from the methyl 9-decenoate GC peak, the GC peak area was not measured. But the presence of methyl (*Z*)-6-decenoate was proved from these results because the MS pattern of methyl 9-decenoate was different from that of methyl

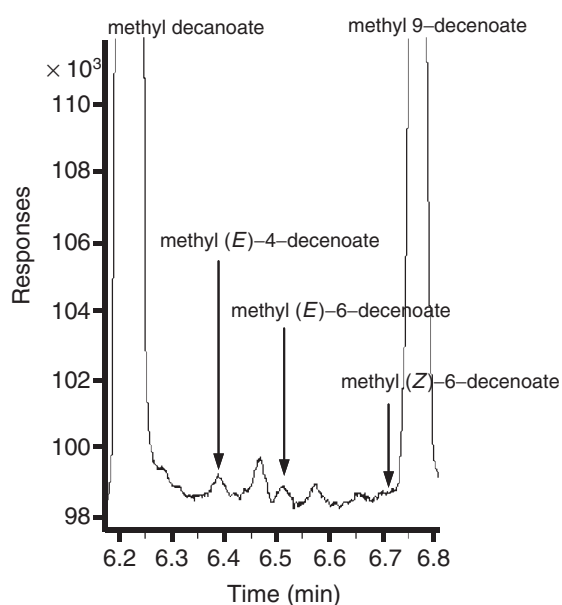
(*Z*)-6-decenoate. The MS,  $m/z$  (relative intensity) data of decenoic acids in heated butter and their methyl esters are listed in Table 2. 5- and 6-Decenoic acids were detected as volatile compounds from red deer. But the geometry of the double bond was not specified.<sup>17)</sup> (*E*)-4-Decenoic acid has not been isolated in nature.

Mechanistically, the formation of decenoic acids such as 4-, 5- and 6-decenoic acids can be considered from  $\delta$ -decalactone, as shown in Fig. 3.

Several  $\delta$ -lactones that have been detected in heated butter and are important to the flavor of butter, are proposed to be formed of  $\delta$ -hydroxy acids or their triglycerides during heat treatment.<sup>5,6)</sup>  $\gamma$ - and  $\delta$ -Decalactone were also observed in the present study (Table 1). It has been reported that the reaction of 4-, 5-, and 6-alkenoic acids with trifluoroacetic acid occurs at 150 °C to afford an equilibrium mixture of corresponding  $\delta$ -,  $\gamma$ -decalactone with the starting material.<sup>18)</sup>

$\delta$ -Decalactone is assumed to transform into (*E*)- and (*Z*)-4-decenoic acid or (*E*)- and (*Z*)-5-decenoic acid *via* the transient formation of carbonium ion with a ring opening in the presence of fatty acids such as acetic acid, in butter. Subsequently, the double bond migrates to afford (*E*)- and (*Z*)-6-decenoic acids *via* the similar process involved in the formation of the carbonium ion.

When  $\delta$ -decalactone was treated with active clay at 150 °C for 5 h in the experiment, (*E*)- and (*Z*)-4-, (*E*)- and (*Z*)-5- and (*E*)- and (*Z*)-6-decenoic acids with  $\gamma$ -decalactone were formed in a ratio of 22:24:25:25:78 (GC peak area). But 9-decenoic acid was not produced. Therefore, the formation of 9-decenoic acid in heated butter did not appear to be due to  $\delta$ -decalactone. It is assumed that 9-decenoic acid was formed from the corresponding triglycerides that exist in butter as a major component.



**Fig. 2.** GC Analysis (6.20–6.71 min) of the Methylated Acidic Fractions of Heated Butter.

**Table 2.** EI Mass Spectra of Decenoic Acids in Heated Butter and Their Methyl Esters

Compound	$m/z$ (relative intensity)
( <i>E</i> )-4-decenoic acid	39(37); 41(87); 54(46); 55(100); 67(82); 68(72); 69(79); 71(64); 81(77); 82(41); 84(54); 95(37); 96(44); 110(87); 123(37); 152(65)
( <i>E</i> )-5-decenoic acid	39(27); 41(67); 43(25); 54(55); 55(100); 67(65); 68(63); 69(65); 81(90); 82(36); 84(45); 95(30); 96(32); 110(71); 123(31); 152(49)
( <i>Z</i> )-5-decenoic acid	39(35); 41(81); 43(29); 54(54); 55(100); 60(26); 67(60); 68(60); 69(59); 81(74); 82(30); 84(38); 95(25); 96(26); 110(53); 152(34)
( <i>E</i> )-6-decenoic acid	39(28); 41(73); 54(30); 55(100); 67(39); 68(40); 69(51); 81(57); 82(22); 83(21); 84(36); 95(26); 96(22); 110(39); 123(26); 152(30)
9-decenoic acid	39(27); 41(62); 55(100); 60(34); 67(27); 68(59); 69(100); 73(39); 81(26); 82(45); 83(49); 84(45); 96(36); 110(72); 123(31); 152(25)
methyl ( <i>E</i> )-4-decenoate	41(48); 43(25); 54(33); 55(53); 59(30); 67(51); 68(33); 69(50); 74(100); 81(38); 82(22); 84(38); 85(42); 96(40); 110(68); 152(42)
methyl ( <i>E</i> )-6-decenoate	41(60); 54(28); 55(82); 67(41); 68(26); 69(44); 74(100); 81(51); 84(60); 87(39); 95(30); 96(38); 110(63); 123(37); 152(52)
methyl ( <i>Z</i> )-6-decenoate	41(61); 54(28); 55(85); 59(36); 67(43); 69(42); 74(100); 81(55); 84(62); 87(38); 95(31); 96(39); 110(66); 123(38); 152(52); 153(28)
methyl 9-decenoate	41(42); 43(18); 55(63); 59(28); 67(17); 68(20); 69(46); 74(100); 82(18); 83(25); 84(32); 87(49); 96(26); 110(44); 135(21); 152(23)

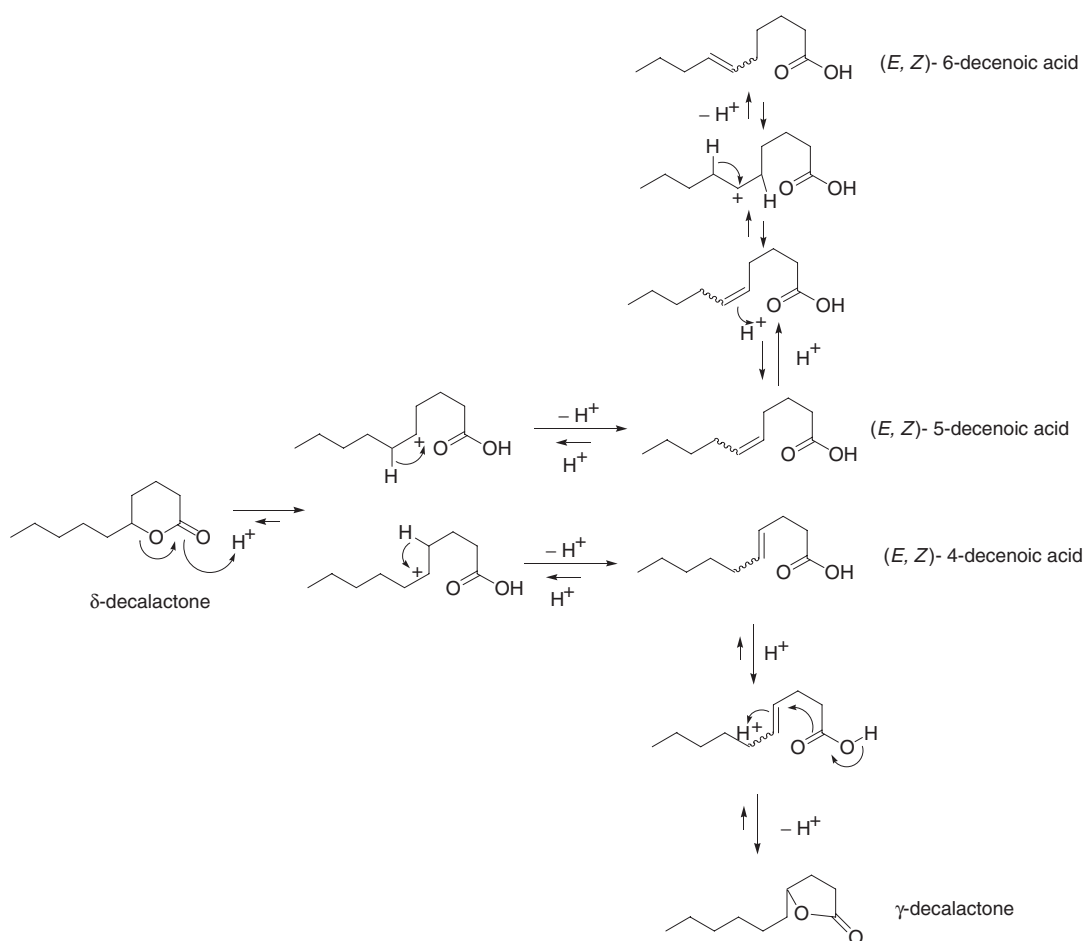


Fig. 3. Formation of Decenoic Acids Such as 4-, 5- and 6-Decenoic Acids in Heated Butter.

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