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ORIGINAL PAPER

Bidirectional Conversion Between 3-Monochloro-1,2-propanediol and Glycidol in Course of the Procedure of DGF Standard Methods

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Abstract NMR observation revealed that bidirectional conversion occurred between 3-monochloropropane-1,2-diol (3-MCPD) and glycidol in the course of the analytical procedure of DFG standard method C-III 18 (09), option A; 3-MCPD was partly converted to glycidol at the transesterification step, and glycidol was converted partly to 3-MCPD at the derivatization step conducted at 80 °C under acidic condition in the presence of NaCl. Based on the proton numbers observed by ^{1}H NMR, the degrees of the conversion were estimated to be 37 and >70%, respectively. In addition, epoxide ring-opening of glycidol and its esters was found to be ca. 90% by the acid treatment described in the method, option B. Thus, it was concluded that the standard method, option A, did not correctly give the combined amount of 3-MCPD esters and glycidyl esters in oils containing glycidyl esters, and the difference of the values obtained by options A and B did not correspond to the amount of glycidyl esters, either. In addition, derivatives of 3-MCPD with phenylboronic acid were not observed by NMR at the derivatization step, although they were detected by GC-MS in the organic phase at the following extraction step.

Keywords 3-Monochloropropane-1,2-diol (3-MCPD) · DFG standard method · Glycidol · Glycidyl ester · NaCl

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Introduction

3-Monochloropropane-1,2-diol (3-MCPD) has recently been a big issue due to the concerns to human health [1, 2]. The recommended guideline for its intake is 2 μg/kg body weight per day. In order to estimate the daily intake from the diet, a quantification method is essential. The amount of 3-MCPD and its fatty acid esters (referred to as esters hereafter) in fat and oil products is currently measured by the standard method established by the German Society for Fat Science (DFG standard methods C-III 18 [3]). In the method, it is stated that the method is not specific to 3-MCPD (esters) and that glycidol and its esters are known to be detected as 3-MCPD. It was thus revised in 2009 to remove them by the acid treatment (option B) prior to the conventional procedure (option A). The values obtained by option B are defined as the true amount of 3-MCPD, whereas the difference between the values obtained by option A and B is defined as the amount of glycidyl esters, since glycidol is considered to be negligible in fats and oils.

Perplexingly, the amounts of glycidyl esters determined in the revised standard method were not consistent with the amounts of those determined directly by the LC-MS method [4] when sample oils spiked with known amount of glycidyl esters were analyzed in our laboratory. The revised standard method is based on the assumption that glycidyl esters were completely detected as 3-MCPD in option A and that the removal of glycidyl esters were complete in option B. However, there was a possibility that the assumption might not be true. Moreover, the mechanism of incorrect detection of glycidyl esters as 3-MCPD by the standard method is not clearly understood. This paper reveals that bidirectional conversion between 3-MCPD and glycidol was observed in the course of the analytical procedure of DFG standard methods C-III 18

(09), and that the method was not suitable for fats and oils which include glycidyl esters.

Experimental Procedures

Materials

3-MCPD, 3-MCPD-d_5, glycidol, phenylboronic acid, sodium methoxide/methanol solution, starting materials for syntheses, and solvents were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). NaCl and acetic acid were purchased from Nakalai Tesque Co. Ltd. (Kyoto, Japan). Soybean oil was the product of Ueda Oils and Fats MFG Co. Ltd. (Kobe, Japan). D_2O (99.96 atom% D) and $CDCl_3$ (99.8 atom% D) were purchased from Aldrich Chemical Company, Inc. (Milwaukee, WI, USA). Other chemicals were of the analytical grade.

Synthesis of Glycidyl Esters

Glycidyl Palmitate

Procedure A

Glycidol (0.27 g, 3.64 mmol) was dissolved in dry pyridine (20 mL) at 0 °C in a three-necked flask equipped with a drying tube. Palmitoyl chloride (1.0 g, 3.64 mmol) was added to the solution in four portions at intervals of 10 min. The reaction mixture was stirred overnight at room temperature, and the solvent was removed by evaporation. The residue was extracted with chloroform (20 mL), and washed with water (20 mL), 0.1 M hydrogen chloride solution (20 mL), sat. sodium hydrogen carbonate solution (20 mL), and finally brine (20 mL). The solvent was evaporated to dryness. The residue was purified by flash chromatography on silica gel (ethyl acetate/hexane = 1/1, vol/vol) to give glycidyl palmitate (0.80 g, 70%) as a white solid and 3-chloro-2-hydroxypropyl palmitate (0.12 g, 9%) as a white solid. Glycidyl palmitate: IR (neat) 2,912, 2,844, 1,737, 1,471, 1,456, 846; ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.40 (dd, J = 3.1, 12.2 Hz, 1H), 3.91 (dd, J = 6.3, 12.2 Hz, 1H), 3.26 (m, 1H), 2.84 (t, J = 4.6 Hz, 1H), 2.65 (dd, J = 2.6, 4.8 Hz, 1H), 2.35 (t, J = 7.6 Hz, 2H), 1.63 (m, 2H), 1.26 (m, 24H), 0.88 (t, J = 6.5 Hz, 3H); ^{13}C NMR (75 MHz, CDCl3) δ 173.5, 64.7, 49.3, 44.6, 30.0, 31.9, 29.6–29.1 (10 × CH_2), 24.8, 22.6, 14.0; MS (ESI) m/z 335.4 $[M + Na]^+$; Anal. Calcd for $C_{19}H_{36}O_3$: C, 73.03; H, 11.61. Found: C, 73.06; H, 11.51. 3-Chloro-2-hydroxypropyl palmitate (3-MCPD palmitate): IR (neat) 3,436, 2,931, 1,737, 1,465, 1,180, 719; ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.22 (d, J = 5.2 Hz, 2H), 3.91 (quint, J = 5.2 Hz, 1H), 3.62 (dd, J = 5.82, 11.3 Hz, 1H), 2.65 (bs, 1H), 2.35 (t, J = 7.5 Hz, 2H), 1.63 (quint, J = 7.4 Hz, 2H), 1.26 (m, 24H), 0.88 (t, J = 6.8 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.1, 68.8, 68.4, 47.8, 33.9, 31.9, 29.6–29.1 (10 × CH_2), 25.1, 22.8, 14.1; MS (ESI) m/z 371.4 $[M + Na]^+$; Anal. Calcd for $C_{19}H_{37}O_3Cl$: C, 65.40; H, 10.69. Found: C, 65.44; H, 10.52.

Procedure B

Palmitic acid (1.0 g, 3.90 mmol) was dissolved in dry methylene chloride (20 mL) at 0 °C under argon atmosphere. 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC·HCl) (0.75 g, 3.90 mmol), *N*,*N*-diisopropylethylamine (DIEA) (0.50 g, 3.90 mmol), and *N*,*N*-dimethylaminopyridine (DMAP) (0.048 g, 0.39 mmol) were added to the solution. The reaction mixture was stirred for 5 min, and then cooled down. Glycidol (0.26 mL, 3.90 mmol) was added to the mixture, and stirred for 10 min, then allowed to warm up to room temperature. After the reaction was complete, the methylene chloride solution was washed with water (20 mL), 0.1 M hydrogen chloride solution (20 mL), sat. sodium hydrogen carbonate solution (20 mL), and finally brine (20 mL). The solvent was evaporated to dryness, and the residue was purified by silica gel column chromatography (ethyl acetate/hexane = 1/1, vol/vol) to give glycidyl palmitate (0.96 g, 79%) as a white solid.

3-Chloro-2-Hydroxypropyl Palmitate (3-MCPD Palmitate)

3-Chloro-2-hydroxy propanol (0.40 g, 3.64 mmol) was dissolved in dry pyridine (20 mL) at 0 °C in a three-necked flask equipped with a drying tube. Palmitoyl chloride (1.0 g, 3.64 mmol) was added to the solution in four portions at intervals of 10 min. The reaction mixture was stirred for 2 h at room temperature. The solvent was then removed by evaporation. The residue was extracted with chloroform (20 mL), washed with water (20 mL), 0.1 M hydrogen chloride solution (20 mL), sat. sodium hydrogen carbonate solution (20 mL), and finally brine (20 mL). The solvent was evaporated to dryness. The residue was purified by flash chromatography on silica gel (ethyl acetate/hexane = 1/2, vol/vol) to give 3-chloro-2-hydroxypropyl palmitate (0.82 g, 64%) as a white solid, 3-chloropropyl 1,2-dipalmitate (0.040 g, 2%) as a white solid, and 3-chloro-1-hydroxypropyl palmitate (0.062 g, 5%) as a white solid. 3-Chloropropyl 1,2-dipalmitate: IR (neat) 2,913, 2,844, 1,728, 1,471, 1,253; ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.22 (m, 1H), 4.28 (dd, J = 4.4, 11.8 Hz, 2H), 3.66 (dd, J = 5.4, 11.8 Hz, 2H), 2.33 (m, 4H), 1.63 (m, 4H), 1.26 (m, 48H), 0.88 (t, J = 6.8 Hz, 6H); ^{13}C NMR

(75 MHz, $CDCl_3$) δ 173.2, 172.8, 70.25, 62.25, 42.2, 34.2, 34.0, 31.9, 29.7–29.1 (20 × CH_2), 24.9, 22.7, 14.1; MS (ESI) *m/z* 609.2 $[M + Na]^+$; Anal. Calcd for $C_{35}H_{67}O_4Cl$: C, 71.57; H, 11.50. Found: C, 71.66; H, 11.45. 3-Chloro-1-hydroxypropyl palmitate: IR (neat) 3,434, 2,922, 1,737, 1,460, 1,177; ^1H NMR (300 MHz, $CDCl_3$) δ 5.06 (m, 1H), 3.83 (d, J = 4.8 Hz, 2H), 3.70 (dd, J = 5.5, 11.5 Hz, 2H), 2.37 (m, 2H), 1.64 (quint, J = 7.5 Hz, 2H), 1.26 (m, 24H), 0.88 (t, J = 6.6 Hz, 3H); ^{13}C NMR δ 173.1, 81.3, 64.2, 44.3, 34.2, 31.9, 29.6–29.1 (10 × CH_2), 25.1, 22.8, 14.1; MS (ESI) *m/z* 371.4 $[M + Na]^+$; Anal. Calcd for $C_{19}H_{37}O_3Cl$: C, 65.40; H, 10.69. Found: C, 65.47; H, 10.47.

3-Propoxypropane-1,2-diol (1-glyceryl 1-propyl ether)

Glycerol (9.7 g, 105 mmol) was dissolved in dry dimethylformamide (100 mL) at 0 °C in a three-necked flask with a condenser. Sodium hydride (60% in oil, washed by *n*-hexane, 0.49 g, 12.2 mmol) was slowly added to the solution. After hydrogen gas was ceased, propyl bromide (1.0 g, 8.13 mmol) was added to the reaction mixture and was stirred for 18 h at 80 °C. After the reaction mixture cooled to the ambient temperature, ethyl acetate (100 mL) was added and was washed with water (100 mL). The aqueous phase was extracted twice with ethyl acetate (50 mL). The organic phase was combined, and was evaporated to concentrate. The resulting residue was purified by open column chromatography (hexane/ethyl acetate/methanol = 4.5:4.5:1, vol/vol/vol) to give 1-glyceryl 1-propyl ether (0.090 g, 8.3%). ^1H NMR (300 MHz, D_2O) δ 3.84 (m, 1H), 3.69–3.40 (m, 7H), 1.55 (sext, J = 7.2 Hz, 2H), 0.85 (t, J = 7.2 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 73.8, 71.8, 71.0, 63.4, 23.1, 10.3; MS (ESI) *m/z* 135.4 $[M + H]^+$; HRMS (ESI) 135.1023 ($C_6H_{15}O_3$ requires 135.1021).

Determination of 3-MCPD Forming Substances by DGF Standard Methods C-III 18 (09)

The contents of 3-MCPD forming substances were determined as described in DGF standard methods C-III 18(09) with a slight modification. Option A: soybean oil (0.1 g) mixed with 3-MCPD was dissolved in 0.5 mL solvent consisted of *tert*-butyl methyl ether and ethyl acetate (=4:1, vol/vol). To the sample, 3-MCPD-d_5 (2 μg) and 0.5 M sodium methoxide/methanol solution (1 mL) was added and left for 10 min at room temperature (step 2). The mixture was extracted using *n*-hexane (3 mL) and water containing 16.7% NaCl and 3.3% acetic acid (3 mL). The aqueous phase was rinsed with *n*-hexane (3 mL, step 3). The aqueous phase was mixed with derivatization reagent (0.125 g/mL phenylboronic acid solution, 0.5 mL) and left at 80 °C for 20 min (step 4). Then, the extraction was conducted using *n*-hexane (3 mL, step 5). The organic phase was collected, evaporated to dryness, and was dissolved again to 2,2,4-trimethylpentane (2 mL). The sample was filtered by paper before it was brought to GC-MS analysis (step 6).

Option B: soybean oils (0.1 g) spiked with glycidyl esters were treated with 0.5% sulfuric acid/propanol solution (0.5 mL) at 45 °C for 15 min in the ultrasonic bath (step 1). The samples were put through the above-mentioned procedure, steps 2–6.

GC-MS

GC-MS was carried out using a GCMS QP 2010 instrument (Shimadzu, Kyoto, Japan) connected to a DB-5 capillary column (30 m, 0.25 μm, Agilent Technologies, Tokyo, Japan). The column temperature was controlled as follows; it was kept at 60 °C for 1 min, raised at 6 °C /min to 190 °C, further raised at 20 °C/min to 280 °C, and kept at 280 °C for 6 min. The temperature of the programmed-temperature vaporizer (PTV) injector was controlled as follows; it was kept at 60 °C for 1 min, raised at 10 °C/min to 180 °C and kept at 180 °C for 20 min. The temperatures of the interface and the ion source were set at 250 and 200 °C. Other conditions for GC-MS were the same with those described in DGF standard methods C-III 18.

LC-MS

Glycidyl esters were treated with 0.5% sulfuric acid/propanol solution (0.5 mL) at 45 °C for 15 min in an ultrasonic bath. To the sample, *n*-hexane (3 mL) and water (3 mL) were added and mixed by vortex. The organic phase was collected and dried over sodium sulfate. It was evaporated to dryness and dissolved in acetonitrile (1.5 mL). The resulting sample was then analyzed by API 2000 LC/MS/MS system (Life Technologies Japan, Tokyo, Japan) connected to a YMC-Triart C18 column (2.0 × 50 mm, S-3 μm, 12 nm, YMC Co. Ltd., Kyoto, Japan). The column temperature was set at 40 °C. Elution was conducted at a flow rate of 0.2 mL/min, using mobile phase A consisting of acetonitrile/methanol/water (=17:17:6, vol/vol/vol) and mobile phase B consisting of 2-propanol. The binary gradient program was as follows; mobile phase A, 98% and B, 2% at 0.0 min; a linear gradient elution to A, 85% and B, 15% from 0.0 to 15.0 min; an isocratic elution with A, 5% and B, 95% from 15.1 to 25.0 min. Mass chromatograms were recorded by a triple stage quadrupole mass spectrometer equipped with an atmospheric pressure chemical ionization (APCI) ionizer. The ion spray voltage was +4,500 V.

NMR

^{1}H- and ^{13}C-NMR spectra were recorded on a JEOL AL-300 spectrometer (Tokyo, Japan) at 300 and 75 MHz, respectively and were referenced to internal tetramethylsilane ($CDCl_3$) or to the residual protonated solvent (for D_2O and methanol-d_4).

Results

DFG standard methods C-III 18 (09) describes that glycidol and its fatty acid esters are detected as 3-MCPD in the conventional procedure (option A) and that they should be removed by acid treatment prior to the conventional procedure (option B). As diagrammatically described in Fig. 1, it consists of the following steps; (1) treatment of oil samples with 0.5% sulfuric acid/propanol to open epoxide ring, (2) transesterification of glycerides and other esters with sodium methoxide/methanol, (3) fractionation of fatty acid esters from 3-MCPD using *n*-hexane/water containing 16.7% NaCl and 3.3% acetic acid, (4) derivatization of 3-MCPD in the aqueous phase with phenylboronic acid, (5) extraction of resulting derivatives with *n*-hexane, and (6) GC-MS analysis. In order to verify the effectiveness of the acid treatment (step 1), glycidyl esters were prepared first.

Synthesis of Glycidyl Fatty Acid Esters

The Schotten-Baumann reaction, which is the one between acid chlorides and alcohols under basic conditions, is generally applicable for fatty acid ester synthesis. Palmitoyl chloride was reacted with glycidol to give not only the corresponding glycidyl ester (70%) but also the unexpected 3-MCPD ester (9%). The epoxide ring of the ester was nucleophilically substituted by the chloride ion which was generated near the epoxide in the reaction. On the other hand, esters are also obtained by the reaction between free fatty acids and alcohols using condensation agents such as carbodiimides under the basic conditions. The corresponding glycidyl ester was afforded as the unique product (79%) using 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC·HCl) as the condensation agent in spite of the chloride ion existing in the reaction system.

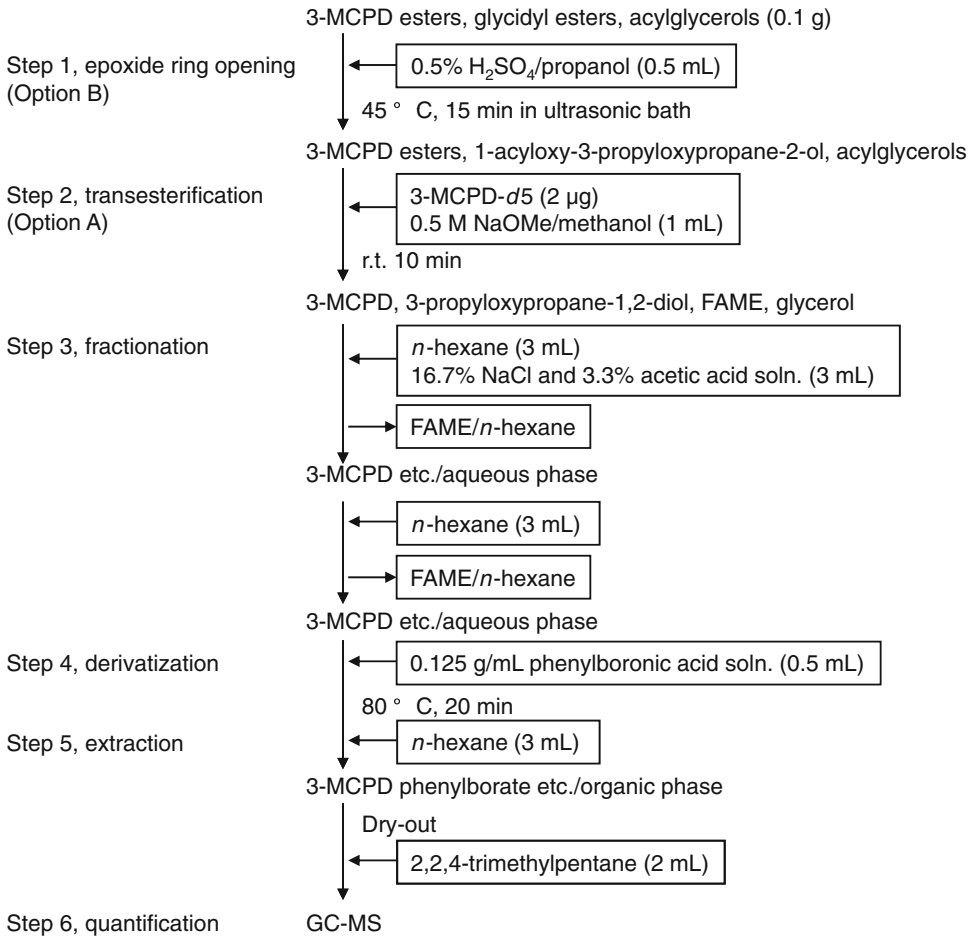

Fig. 1 Flow diagram of DGF standard methods C-III 18. Major steps, reaction conditions, and conversions of target compounds were described

Therefore, the 3-MCPD ester formation by Schotten-Baumann reaction progresses concurrently with the glycidyl ester formation.

Palmitoyl chloride was reacted with 3-MCPD to give the monoester of primary alcohol (3-MCPD ester, 64%), one of the secondary alcohols (3-MCPD ester isomer, 5%), and the diester (3-MCPD diester, 2%). This result suggested that the first esterification took place at the less hindered 1-position hydroxyl group. Interestingly, no glycidyl ester production was observed in the EDC·HCl system. The glycidyl ester did not form due to too low basicity of pyridine to deprotonate from the 2-position hydroxide. Thus, the deprotonation may cause the conversion of 3-MCPD ester to glycidyl ester, and the nucleophilicity of the chloride ion may cause the conversion of the glycidyl ester to the 3-MCPD ester.

Evaluation of Acid Treatment

The resulting glycidyl esters were used to verify the effectiveness of the acid treatment (step 1). Soybean oil spiked with glycidol or glycidyl esters were treated according to the standard method, option B, which included the treatment with 0.5% sulfuric acid/propanol solution (Table 1). 3-MCPD was detected by GC-MS in the oil samples spiked with $\geq$10 ppm of glycidols but was not in that with 5 ppm. Similar results were obtained with oils spiked with glycidyl palmitate and oleate. These results indicated that the removal of glycidol in addition to its esters were incomplete. The glycidyl esters of $\leq$5 ppm might be reduced to undetectable amount, but should remain still in the samples after the acid treatment. The observation was consistent with the observation of Shimizu et al. [5], though they did not directly quantify the residual glycidyl esters.

Table 1 Detection of 3-MCPD in oils spiked with glycidol or glycidyl esters by DGF standard methods C-III 18(09), option B

Spiked compound	Spiked amount (ppm)	Detected amount as 3-MCPD[a] (ppm)
Glycidol	5	n.d.[b]
	10	1.17 ± 0.44
	20	1.68 ± 0.65
Glycidyl palmitate	5	n.d.[b]
	10	0.64 ± 0.23
	20	1.04 ± 0.11
Glycidyl oleate	5	n.d.[b]
	10	0.63 ± 0.17
	20	1.31 ± 0.30

Soybean oil was spiked with glycidol or its esters. The oil samples were treated as described in DGF standard method C-III 18(09), option B

[a] The amount in soybean oil without any spike was 0.32 ppm. The amount was subtracted from those detected in the spiked oil samples. All analyses were conducted 3–4 times. The mean values and the standard deviations were presented

[b] Not detected. The minimum limit of detection was 0.2 ppm

In order to confirm the incomplete ring-opening of epoxides, the residual glycidyl esters after the acid treatment were directly measured by LC-MS. As shown in Table 2, 0.11, 0.42, and 1.72 ppm of glycidyl palmitate remained in the oils spiked with 1, 5, and 20 ppm, even after the acid treatment. Likewise, removal of glycidyl oleate was not completed by the acid treatment. The residual ratio was 10% approximately. Therefore, the epoxide ring-opening of glycidyl esters was confirmed to be ca. 90% by the acid treatment.

Possibility of 1-Glyceryl 1-Propyl Ether Causing the Incorrect Detection of 3-MCPD

The treatment of glycidyl esters by sulfuric acid/propanol gives the corresponding 1-acyloxy-3-propyloxypropane-2-ol. Here, 1-acyloxy-propane-2,3-diol (monoacylglycerol) is not expected due that sulfuric acid/propanol contains little water. Based on the result above, 90% of glycidyl esters should be converted to 1-acyloxy-3-propyloxypropane-2-ol. Its deacylation by the following treatment with sodium methoxide (step 2) gives 3-propyloxypropane-1,2-diol (1-glyceryl 1-propyl ether). In general, 1-glyceryl 1-propyl ether is stable under basic conditions. However, glycidol, which is responsible for the incorrect detection as 3-MCPD, could be generated from the ether by the attack of the neighboring 2-position alkoxide on the epoxide-carbon if the alkoxide is generated under basic conditions. In order to investigate the possibility of 1-glyceryl 1-propyl ether causing the incorrect detection of 3-MCPD, the compound was synthesized, and its behavior under treatment with sodium methoxide was monitored by NMR. ^{13}C-NMR spectra were identical before and after the treatment

Table 2 Residual amount of glycidyl esters after treatment with 0.5% sulfuric acid/propanol

Compound	Amount (ppm)	Detected amount[a] (ppm)	Residual ratio (%)
Glycidyl palmitate	1.0	0.11	11.5
	5.0	0.42	8.5
	20.0	1.72	8.6
Glycidyl oleate	1.0	0.11	10.6
	5.0	0.52	10.3

Glycidyl esters were treated with 0.5% sulfuric acid/propanol solution at 45 °C for 15 min in the ultrasonic bath as described in DGF standard method C-III 18(09), option B

[a] Glycidyl esters were analyzed by LC-MS

(Fig. 2), since the peak at 49 ppm belonged to methanol. It was thus clarified that glycidol was not generated by the sodium methoxide treatment of 1-glyceryl 1-propyl ether. Consequently, the acid treatment of 1-acyloxy-3-propyloxypropane-2-ol (step 1) did not cause the incorrect detection of 3-MCPD by the standard procedure, option B.

Bidirectional Conversion of 3-MCPD and Glycidol

When soybean oil (0.1 g) spiked with 3-MCPD (1 μg) was treated according to the DGF standard method, option A (without acid treatment, steps 2–6), the peak area of phenylboronic acid derivatives of 3-MCPD detected by GC-MS was 600. On the other hand, it was 2,600 when the sample was treated in the same procedure without the transesterification step. The peak areas of 3-MCPD-d_5, the internal standard, were 1,100 and 4,800 with or without transesterification. It was therefore indicated that 77% of 3-MCPD was lost somehow due to the step. Weisshaar estimated that sodium methoxide might decompose 3-MCPD [6]. However, the details of the decomposition have not yet been understood.

Thus, the behavior of 3-MCPD under the transesterification step was directly monitored by ^{1}H and ^{13}C NMR. 3-MCPD was dissolved in sodium methoxide/methanol solution, kept at room temperature for 10 min. After the addition of D_2O, the sample was analyzed by NMR measurement. The peaks derived from 3-MCPD (71.1, 62.5, 45.9 ppm, Fig. 3a) were the only peaks observed before the treatment. After the treatment, the peaks derived from glycidol (61.5, 53.0, 45.0 ppm) appeared on the ^{13}C-NMR chart (Fig. 3b). The conversion ratio was 37% calculated on their proton numbers obtained by ^{1}H NMR.

The behavior of glycidol in the standard method option A was also monitored by NMR. Glycidol was treated in the similar way to the standard method steps 2–4 (transesterification to derivatization steps), except *n*-hexane and phenylboronic acid was not added and D_2O was used instead of water (details of reaction conditions were given in the legend Fig. 3d). pH of the reaction mixture was 4.2. In addition to the peaks derived from glycidol, those from 3-MCPD were newly observed by NMR after the treatment (Fig. 3c, d). The conversion ratio from glycidol to 3-MCPD was 70%, when it was calculated on their proton numbers. Interestingly, the conversion was not detected when the derivatization step (step 4) was conducted at room temperature instead of 80 °C. On the other hand, the conversion increased to nearly 100% when glycidol was directly dissolved in D_2O containing 16.7% NaCl and 3.3% acetic acid (pH 1.9), and stood at 80 °C for 20 min. It was thus indicated that the heating under the acidic conditions at the derivatization step accelerated the conversion of glycidol to 3-MCPD greatly.

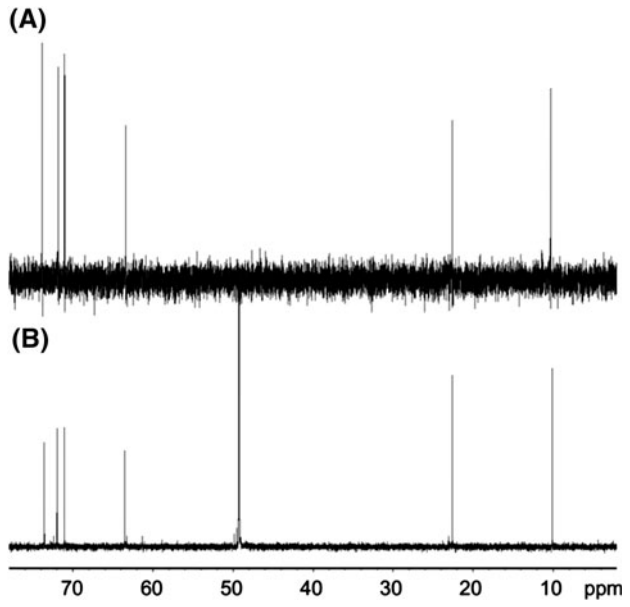

Fig. 2 ^{13}C-NMR spectra of 3-propoxypropane-1,2-diol. **a** 3-propoxypropane-1,2-diol (standard) dissolved in D_2O; **b** 3-propoxypropane-1,2-diol (0.01 g) was dissolved in 0.5 M sodium methoxide/methanol solution (0.2 mL) and kept at room temperature for 10 min. Then, D_2O (0.6 mL) was added to the solution. All reactions were conducted in NMR tubes

Production of 3-MCPD Derivatives at the Derivatization Step

As shown Fig. 3d, treatment of glycidol with steps 2–4 without phenylboronic acid generated 3-MCPD with 70% of conversion. The treatment was then conducted with phenylboronic acid. The reaction conditions were the same with the standard method steps 2–4 except *n*-hexane was not added and D_2O was used instead of water. The conversion of glycidol to 3-MCPD was again observed by ^{13}C NMR (Fig. 4b). The degree of conversion was 74% calculated on the proton numbers observed by ^{1}H NMR. Suppose that the errors of integration value measured in ^{1}H NMR were ±5%, the conversion ratio were nearly the same with or without phenylboronic acid at the derivatization step. On the other hand, the 3-MCPD phenylborate was not detectable (Fig. 4b). Association constant of phenylboronic acid and diols was reported to drastically change at pH ~7.5, and the phenyl borate was hardly formed below pH 6.5 [7]. It was therefore speculated that the 3-MCPD phenyl borate were produced only in a small amount in the experimental conditions of pH 4.2, and thus were undetectable by NMR.

The reaction mixture was further extracted by *n*-hexane as the procedure step 5. After the removal of the hexane phase, the aqueous phase was analyzed by NMR again. Both of glycidol and 3-MCPD were observed by ^{13}C NMR

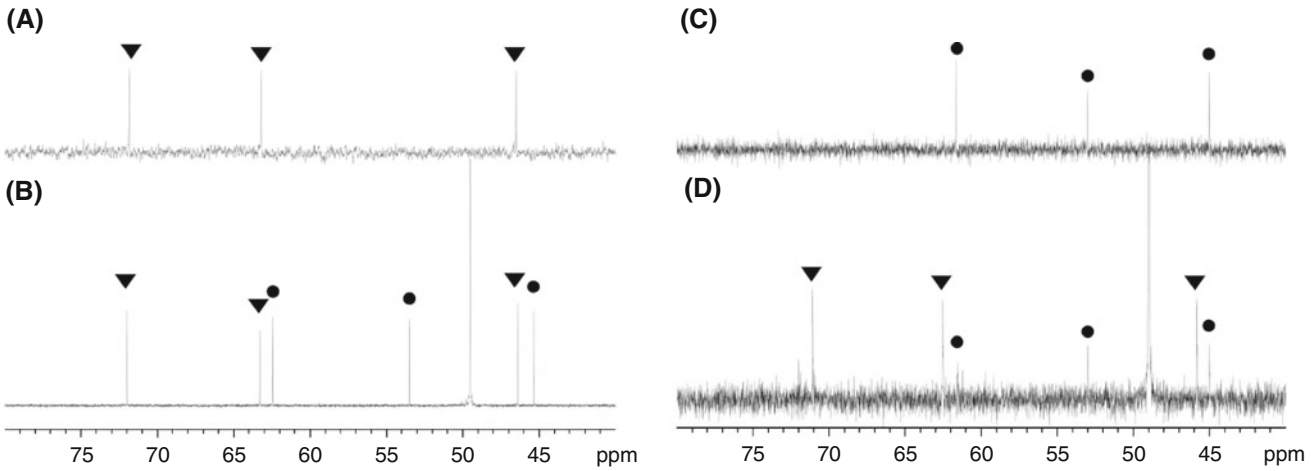

Fig. 3 ^{13}C-NMR spectra of 3-monochloropropane-1,2-diol (3-MCPD, *inverted triangles*), glycidol (*closed circles*), and their products. **a** 3-MCPD (standard) dissolved in D_2O; **b** 3-MCPD (0.13 g) dissolved in 0.5 M sodium methoxide/methanol solution (1 mL) was kept at room temperature for 10 min. Then, D_2O (1 mL) was added to the solution. **c** glycidol (standard) dissolved in D_2O; **d** glycidol (0.01 g) was dissolved in 0.5 M sodium methoxide/methanol solution (0.2 mL) and kept at room temperature for 10 min. Then, D_2O containing 16.7% NaCl and 3.3% acetic acid (0.6 mL), was added to the mixture, and allowed to stand at 80 °C for 20 min.; All reactions were conducted in NMR tubes

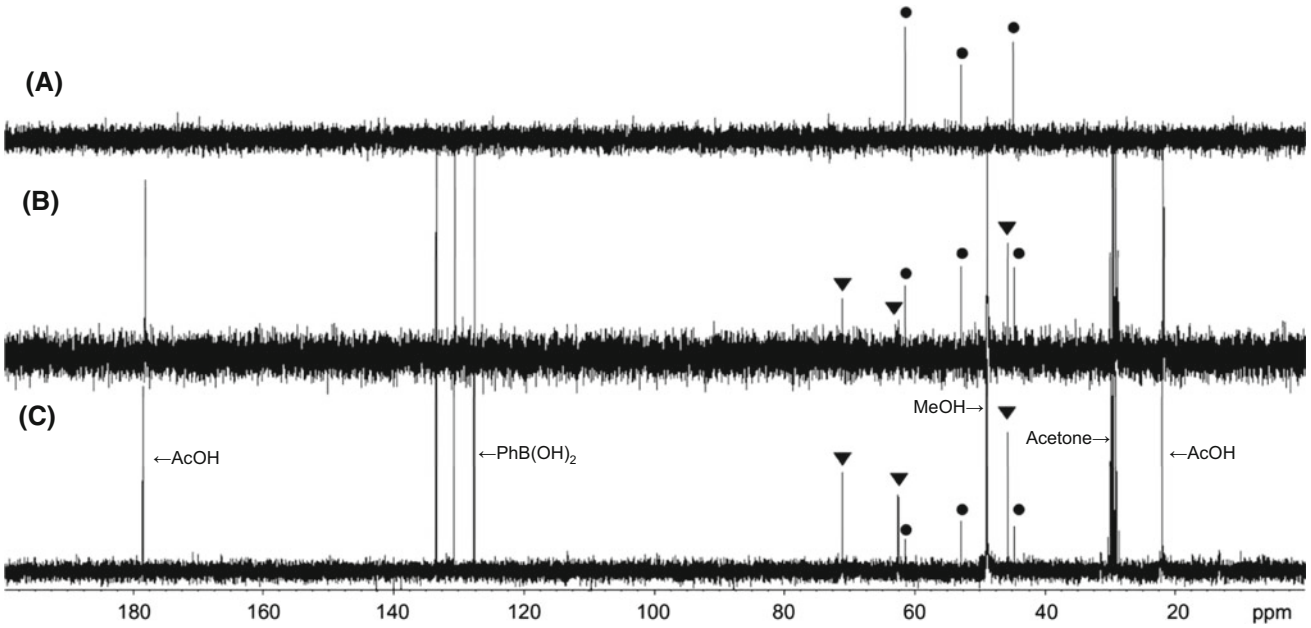

Fig. 4 The conversion of glycidol to 3-MCPD under the condition of option A. The *circles* and *triangles* represent the peaks of glycidol and 3-MCPD, respectively. **a** Glycidol (standard) dissolved in D_2O; **b** glycidol (0.01 g) was dissolved 0.5 M sodium methoxide/methanol solution (0.2 mL) and kept at room temperature for 10 min. Then, D_2O containing 16.7% NaCl and 3.3% acetic acid (0.6 mL) and of acetone-d_6 containing 12.5% (w/v) phenylboronic acid (0.133 mL) were added to the mixture, and allowed to stand at 80 °C for 20 min.; **c** After treatments described in **b**, the reaction mixture was washed by hexane (1 mL). The resulting aqueous phase was analyzed; all reactions were conducted in NMR tubes

(Fig. 4c), with the ratio of 13:87, calculated from the proton numbers obtained by ^{1}H NMR. These results indicated that the extraction of 3-MCPD phenyl borate, which was more hydrophobic than 3-MCPD, to the organic phase shifted the equilibrium of the three compounds, namely 3-MCPD phenyl borate, 3-MCPD, and glycidol in the aqueous phase. As a result, the ratio of 3-MCPD against glycidol increased from 74 to 87%. It should be noted that glycidol and 3-MCPD remained in the aqueous phase even after the hexane extraction at step 5 of the standard method.

The low efficiency of the 3-MCPD phenyl borate formation and extraction might explain the relatively high standard deviations given in Table 1.

Discussion

It has been described that the epoxide ring-opening in glycidol and its esters was incomplete by the acid treatment described in the DGF standard methods C-III 18 (09), option B, and that the bidirectional conversion between 3-MCPD and glycidol was observed by NMR in the course of the method. The behaviors of 3-MCPD esters and glycidyl esters, which were supposed to be in fats and oils, in the course of the standard method are schematically shown in Fig. 5. 3-MCPD produced by the transesterification using sodium methoxide was partly converted to glycidol in the step (37%). There also is a possibility that 2-MCPD were converted to glycidol, though it has to be proven. The resulting glycidol, in addition to glycidol derived from glycidyl esters, were partly converted to 3-MCPD in the following steps, which were conducted in the presence of saturated NaCl under acidic conditions at 80 °C (74%). Glycerol was not generated from glycidol under the conditions, which could be explained that there

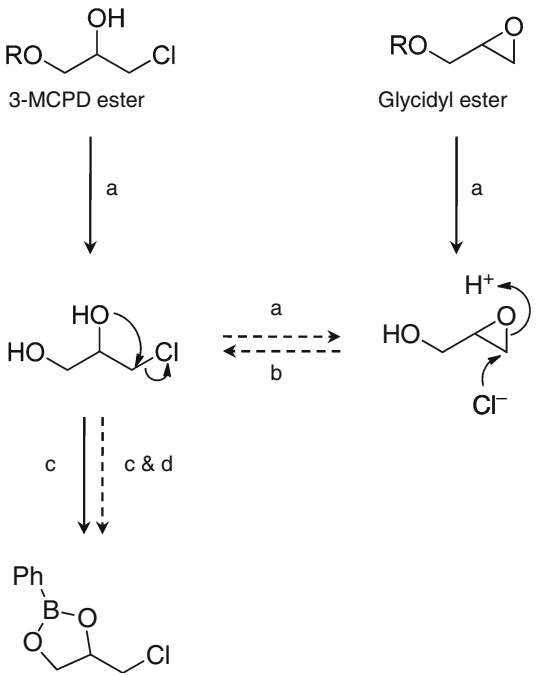

Fig. 5 Behaviors of 3-MCPD esters and glycidyl esters under the procedure of DGF standard method C-III 18(09). *R* represents fatty acyl group. *Arrows with solid lines* represent the conventionally known/believed routes, whereas those with *dotted lines* represent routes newly confirmed in this study. Reagents and conditions: **a** Sodium methoxide/methanol, rt, 10 min. **b** Acetic acid, NaCl, water. **c** Phenylboronic acid, 80 °C, 20 min. **d** Hexane extraction

were abandoned chloride ions with high nucleophilicity, and few hydroxyl ions in the solution. What was important was that the degree of conversion from glycidol to 3-MCPD depended on the conditions of the procedure steps 2–5 (transesterification, derivatization, and extraction), and was not 100%. This observation contradicted that of Kuhlmann cited in Ref. [8] namely that the conversion was nearly complete. Based on our observation, the standard method, option A, did not give the combined amount of 3-MCPD esters and glycidyl esters correctly. The removal of epoxides by the acid treatment described in option B was not complete either, as shown in Table 2. Therefore, the difference of the values obtained by options A and B did not correspond to the amount of glycidyl esters.

The conversion of 3-MCPD to glycidol at the transesterification step was estimated to be 37% by NMR, whereas that of glycidol to 3-MCPD at the following steps was 74%. The loss of 3-MCPD in total was thus ca. 10%, which was not consistent with the observation described in the section 'bidirectional conversion of 3-MCPD and glycidol', where the loss was estimated to be 77% under the influence of the transesterification step. In another report of ours, it was clarified that the loss was also caused by the low extraction capability of *n*-hexane used in the extraction step (step 5) [9]. The substitution of *n*-hexane with a more polar solvent such as chloroform increased the recovery of 3-MCPD derivatives. In the mean time, the derivatives of 3-MCPD with phenylboronic acid were not observed in NMR analysis in the aqueous phase at the derivatization step (step 4), although they were detected by GC-MS in the hexane phase obtained in step 5. It was thus estimated that the derivatives were rather formed in the course of hexane extraction (Fig. 5), than at the derivatization step, and the polarity of the solvent might affect the production as well as the recovery of the derivatives.

Conclusion

This paper reports that bidirectional conversion was confirmed between 3-MCPD and glycidol in the course of the analytical procedure of DFG standard methods C-III 18 (09), option A; 3-MCPD was partly converted to glycidol at the transesterification step, and glycidol was converted partly to 3-MCPD at the derivatization step conducted at 80 °C under acidic condition in the presence of NaCl. In addition, epoxide ring-opening of glycidol and its esters was shown to be incomplete by the acid treatment described in the method, option B. Thus, the standard method, option A, did not give the combined amount of 3-MCPD esters and glycidyl esters correctly, and the difference between the values obtained by options A and B did not correspond to the amount of glycidyl esters, either. The

restricted application of the standard method, option A, to glycidyl ester-free samples is recommended. In addition, the conversion of 3-MCPD to phenylboronic acid was not observed by NMR at the derivatization step. The derivatization was estimated to occur rather in the following hexane extraction step. The observations presented in this paper are important for our understanding of the standard method, and for the interpretation of the values so far given by the standard method.

References

1. Food Advisory Committee (2000) Genotoxicity of 3-monochloropropane-1,2-diol, FdAC/Contaminants/48. Paper for Discussion. UK Food Standards Agency, London. http://archive.food.gov.uk/pdf_files/papers/fac_48.pdf. Accessed Oct 2010
2. FAO/WHO (2001) Joint FAO/WHO Expert Committee on Food Additives (JECFA) Fifty-Seventh Meeting, Rome, 2001, Summary and conclusions (Rome: Food and Agriculture Organization of the United Nations/World Health Organization). http://www.vegsource.com/articles/carrageenan/JECFA_2001_Carrageenan_Report.pdf. Accessed Oct 2010
3. (2009) Ester-bound 3-chloropropane-1,2-diol (3-MCPD esters) and glycidol (glycidol esters). Deutsche Gesellschaft fuer Fettwissenschaft: DGF Standard Methods (14. Supplement) C-III 18
4. Masukawa Y, Shiro H, Nakamura S, Kondo N, Jin N, Suzuki N, Ooi N, Kudo N (2010) A new analytical method for the quantification of glycidol fatty acid esters in edible oils. J Oleo Sci 59:81–88
5. Shimizu M, Kudo N, Shiro H, Yasunaga K, Masukawa Y, Katsuragi Y, Yasumasu T (2010) Comparison of indirect and direct quantification of glycidol fatty acid ester in edible oils. J Oleo Sci 539:535–539
6. Weisshaar R (2008) Determination of total 3-chloropropane-1, 2-diol (3-MCPD) in edible oils by cleavage of MCPD esters with sodium methoxide. Eur J Lipid Sci Technol 110:183–186
7. Yan J, Springsteen G, Deeter S, Wang B (2004) The relationship among pKa, pH, and binding constants in the interactions between boronic acids and diols. Tetrahedron 60:11205–11209
8. Weisshaar R, Perz R (2010) Fatty acid esters of glycidol in refined fats and oils. Eur J Lipid Sci Technol 112:158–165
9. Kaze N, Sato H, Yamamoto H, Watanabe Y Improvement of accuracy in quantification of 3-monochloropropane-1,2-diol and its esters by DGF standard methods C-III 18 (in submission)