Notes

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γ-Induced Addition of Thiols to Methyl Oleate

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γ-Induced addition reactions of thiols to methyl oleate were investigated and the corresponding sulfides were prepared in yield of 94—97%. The adducts consisted of approximately equal amount of C-9-adduct and C-10-adduct. The effects of concentration of ethanethiol, dose and solvent were examined. Diethyl disulfide and sulfide also afforded the ethanethiol adducts in lower yield.

Addition reactions of thiols and hydrogen sulfide to olefins are extensively investigated; light,[2-4] heat,[5,6] radiations,[7-11] and chemicals[12] are used as a reaction initiator and many sulfides have been synthesized. However, most of the works are concentrated on the olefins which are relatively small molecules having a double bond at the 1- or 2-position. As a part of the series of radiation chemical studies on the fatty acid esters, we investigated γ-induced addition reactions of thiols to methyl oleate (MO). In the case of higher unsaturated fatty acid, no investigation on the γ-induced addition of thiol has been undertaken. This paper describes structures of alkylthio derivatives of methyl stearate formed by γ-irradiation of MO in the presence of thiols. The effects of radiation dose, sulfide, disulfide and solvent on the addition of ethanethiol (EtSH) were also examined.

The mass spectrum of EtSH-adducts is shown in Fig. 1. The molecular ion of *m/e* 358 shows 1: 1 adduct of EtSH to MO. The data of elemental analysis are also in good agreement with those of the 1: 1 adduct of them. The structure of the adduct is presumed to be sulfide type from the fact this compound does not reduce iodine solution. In addition, characteristic fragment peaks which indicate the scission at the bonds shown in Fig. 1 are seen at *m/e* 187, 201, 231 and 245. This fragmentations are similar to those of methyl 9- and 10-methoxystearates[13] and methyl 9,10-dimethoxystearate.[14] We determined the molar ratio of C-9-adduct to C-10-adduct to be 1: 1 from the fact that both ratios of peak intensity of *m/e* 187 to 201 and *m/e* 231 to 245 are approximately 1. This consisted with the information obtained upon the additions of benzenethiol to 2-alkenes[10] and that of MeOH to MO.[13]

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The variation of the yields of adducts and recovery of MO in the several molar ratios of EtSH to MO were examined. As shown in Table I, the adducts are obtained in 94—95% yields on the basis of MO when the molar ratios are over 1.2.

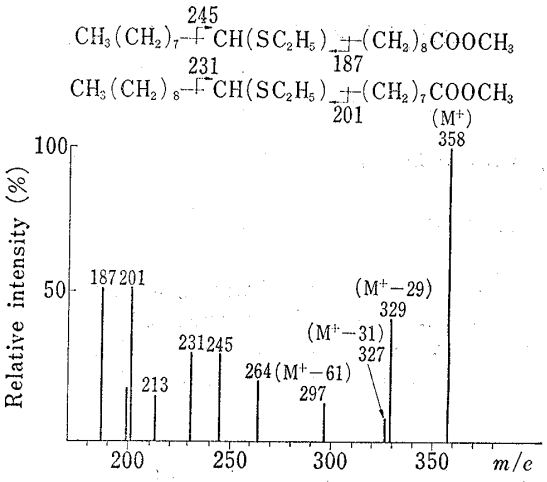

Fig. 1. Illustrative Mass Spectrum of Ethanthiol Adducts of Methyl Oleate

The effect of radiation dose on the yields of adducts were examined by using EtSH. As shown in Table II, no remarkable changes in the yields were observed at the dose range of $0.5-3\times10^7$ rad when the molar ratio is 2.0, however the yields gradually decreased with increasing of dose over 5×10^7 rad. This is explained as a result of radiolysis of adducts which is decomposed in 7, 12 and 24% at dose 5, 10 and 20×10^7 rad, respectively. Oleic acid was also used for the comparison of the addition behavior with that of MO. A little lower % yields than those of methyl ester were observed at any dose in the same molar ratio.

The addition behavior of diethyl disulfide and diethyl sulfide, from which the formation

Table I. Effect of Concentration of EtSH on the Addition Reaction

Molar ratio (EtSH/MO)	Yield of adducts		Recovery of MO
	%	G-value	%
0	—	—	86.4
0.25	13.8	8	73.6
0.5	27.0	15	61.2
1.0	85.5	47	2.7
1.2	94.3	52	1.5
2.0	94.8	53	1.5

dose: 5×10^7 rad

Table II. Effect of Radiation Dose on the Addition of EtSH to MO and Oleic Acid(OA)

Dose $\times10^7$ rad	Yield of adduct, % (G-value)			
	Molar ratio (EtSH/MO)		Molar ratio (EtSH/OA)[a]	
	1.0	2.0	1.0	2.0
0.5	76.7(420)	96.0(530)	51.4(300)	93.1(530)
0.7	79.8(320)	96.2(390)	70.5(290)	94.0(390)
1.0	85.2(240)	97.8(270)	76.4(220)	93.5(270)
3.0	87.2(80)	96.1(89)	78.4(75)	91.0(87)
5.0	85.5(46)	94.8(53)	76.9(44)	88.9(51)
7.0	83.2(33)	93.0(37)	75.0(31)	86.2(35)
10.0	81.0(22)	90.8(25)	73.8(21)	83.2(24)

a) determined after esterification with diazomethane

of $C_2H_5S\cdot$ may be expected, was examined. As is shown in Table III, the yields of adducts from diethyl disulfide increased with increasing of dose. On the other hand, small amounts of the adducts were obtained in the case of diethyl sulfide. The disulfide produced by oxidation of EtSH during the irradiation may be responsible for the addition at the higher doses.

Yields of the adducts in the presence of *n*-hexane, benzene and MeOH are shown in Table IV. No remarkable effects were observed in the addition reactions except for the case of lower molar ratio. From the data on the addition in the presence of MeOH, it was proved

Table III. Yields of Methyl 9- and 10-Ethylthiostearates prepared from Diethyl Sulfide and Diethyl Disulfide

Samples[a]	Yield of Methyl 9- and 10-ethylthiostearates, % Dose ($\times 10^7$ rad)				
	5.0	7.0	10	21	31
MO+Diethyl sulfide	0	0	0.2	0.3	0.3
MO+Diethyl disulfide	1.2	2.2	6.7	9.8	9.4

a) concentration of diethyl sulfide and disulfide: 5%

that the addition of thiol proceeded prior to that of MeOH. Benzene showed no protective effect on the addition of thiol under the conditions used. It is presumed that the solvents used in this experiment can be applied to the preparation of adducts of other thiols which are insoluble in MO.

Table IV. Effect of the Solvents on the Yields of Methyl 9- and 10-Ethylthiostearates (1) and Methyl 9- and 10-Methoxystearates (2)

Yield %	Molar ratio (EtSH/MO)	Concentration of solvent %	No addition	*n*-Hexane	Benzene	MeOH
(1)	1.6	20	94.8	93.2	94.9	94.5
	0.25	5	19.0	20.8	19.1	19.2
(2)	1.6	20	—	—	—	—
	0.25	5	0.2	0.1	0.1	0.7

dose: 5×10^7 rad

From the fact that G-values of the adducts under the experimental conditions are about 20—500, this addition reaction may proceed by radical chain mechanism as well as the reaction proposed by Araki[11] and Sawada, *et al.*[7,8] On the other hand, other thiols such as *n*-propanethiol, isopropanethiol and *n*-butanethiol were irradiated with MO in a similar manner in order to examine the effect of alkyl group of thiols. No difference in their addition behavior was observed and analogous sulfides corresponding to each thiols were obtained in good yields.

From these results, it is concluded that the addition reaction of thiols to MO is interesting not only for a synthetic method of alkylthio derivatives of stearate, but for a subject in radiation chemistry.

Experimental

Irradiation——Samples were sealed in glass ampoules (0.3 or 20 ml) without deaeration and were irradiated with a 45KCi ^{60}Co γ-source at the dose rate of 2.7×10^6 rad/hr.

Materials——MO (99%) obtained from Sigma Chemical Co., Ltd. Oleic acid (Tokyo Kasei Co., Ltd.) was purified by fractional distillation and used a fraction of 98% purity. Thiols obtained from Nakarai Chemical Co., Ltd. were purified by distillation. The other chemicals used were of analytical reagent grade and were used without further purification.

Analysis and Equipment——Gas chromatographic analyses (GC) of products were carried out with a Shimadzu GC-4APF equipped with hydrogen flame detectors and 3 m $\times$ 4 mm glass columns packed with 25% diethylene glycol succinate. Methyl myristate was used as a internal standard, and the quantitative analyses of reaction products were carried out by GC unless otherwise noted. The NMR spectra were measured with a JEOL JNM-C-60H spectrometer. The mass spectra were obtained with a JEOL JMS-01SG. The IR spectra were measured with a JASCO DS-301 infrared spectrophotometer from 4000—650 cm^{-1} as a neat.

Addition of Thiols to MO——A mixture of MO (0.02 mole) and thiols (0.04 mole) was irradiated to a total dose 5×10^6 rad, and the products were purified by distillation.

Methyl 9- and 10-ethylthiostearates: bp 193—195° (3 mmHg), *Anal.* Calcd. for $C_{21}H_{42}O_2S$: C, 70.33; H, 11.80; S, 8.94. Found: C, 70.61; H, 11.87; S, 8.95. IR ν_{max} cm^{-1}: 2920, 2850, 1740, 1465, 1435, 1370, 1250, 1205, 1175, 720. NMR (CCl_4) τ: 6.38 (s, $COOCH_3$), 7.35—7.85(m), 8.00—8.90(m), 9.10(t).

Methyl 9- and 10-*n*-propylthiostearates: Yield 94.4%, bp 202° (3 mmHg), *Anal.* Calcd. for $C_{22}H_{44}O_2S$: C, 70.90; H, 11.90; S, 8.60. Found: C, 71.01; H, 11.92; S, 8.45. Mass Spectrum *m/e* (relative intensity, %): 372 (98.0, M^+), 329 (100, $M^+-C_3H_7$), 259 (66.0, $[C_3H_7SCH(CH_2)_8COOCH_3]^+$), 245 (66.7, $[C_3H_7SCH(CH_2)_7COOCH_3]^+$), 215 (98.6, $[CH_3(CH_2)_8CHSC_3H_7]^+$), 201 (99.0, $[CH_3(CH_2)_7CHSC_3H_7]^+$).

Methyl 9- and 10-isopropylthiostearates: Yield 93.9%, bp 196° (3 mmHg), *Anal.* Calcd. for $C_{22}H_{44}O_2S$: C, 70.90; H, 11.90; S, 8.60. Found: C, 70.62; H, 11.99; S, 8.75. Mass Spectrum *m/e*: 372 (48.1, M^+), 329 (100, $M^+-C_3H_7$), 259 (43.5, $[C_3H_7SCH(CH_2)_8COOCH_3]^+$), 245 (43.2, $[C_3H_7SCH(CH_2)_7COOCH_3]^+$), 215 (60.5, $[CH_3(CH_2)_8CHSC_3H_7]^+$), 201 (59.5, $[CH_3(CH_2)_7CHSC_3H_7]^+$).

Methyl 9- and 10-*n*-butylthiostearates: Yield 95.1%, bp 213° (3 mmHg), *Anal.* Calcd. for $C_{23}H_{46}O_2S$: C, 71.44; H, 11.99; S, 8.29. Found: C, 71.37; H, 11.84; S, 8.15. Mass Spectrum *m/e*: 386 (100, M^+), 329 (100, $M^+-C_4H_9$), 273 (64.0, $[C_4H_9SCH(CH_2)_8COOCH_3]^+$), 259 (64.7, $[C_4H_9SCH(CH_2)_7COOCH_3]^+$), 229 (87.2, $[CH_3(CH_2)_8CHSC_4H_9]^+$), 215 (88.1, $[CH_3(CH_2)_7CHSC_4H_9]^+$).

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Binding of Calcium by Soluble Fraction from Normal Rat Liver

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The calcium-binding activity in the soluble fraction of a normal rat liver was studied. Calcium binding in the supernatant depends on the competition between a cation-exchange resin and a soluble calcium-binding substance for added calcium. The calcium concentration found in the supernatant of the test system was approximately 12-fold of that of calcium contained in the soluble fraction of a normal rat liver. The binding of calcium in the supernatant of the test system using the heat-treated soluble fraction increased linearly up to 0.75 mM calcium and was saturated 1.0 mM calcium. When the radiocalcium-binding activity was expressed as *S/R* net, the radiocalcium-binding activity existed in soluble fraction, and it increased approximately 4-fold by the heat treatment. These results suggest that the calcium-binding factor exists in the soluble fraction of a normal rat liver.

A calcium-binding protein (CaBP) was first reported to be present in the duodenal mucosa of vitamin D-treated rachitic chicks by Wasserman and Taylor.[2)] A similar protein has been found in the small intestine of a number of mammals including the rat, dog, pig, cow, monkey, and human.[3)] Vitamin D-dependent CaBP has also been identified in the kidney of which

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