[Chem. Pharm. Bull. 24(4) 756—762 (1976)]

UDC 547.39'26.09 : 615.277.3.076.9

Chemical and Biochemical Studies on Carbohydrate Esters. III.[1] Antitumor Activity of Unsaturated Fatty Acids and Their Ester Derivatives against Ehrlich Ascites Carcinoma[2]

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(Received July 16, 1975)

Antitumor activity of some unsaturated fatty acids and the ester derivatives was tested with Ehrlich ascites carcinoma in mice. The compounds examined were A) 10-undecenoic, elaidic, oleic, linoleic, and linolenic acids, B) methyl esters of oleic, linoleic, and linolenic acids, C) 1-O-acyl-β-D-glucopyranose tetraacetates derived from oleic, linoleic, and linolenic acids, and D) sucrose monoesters prepared from the fatty acids belonging to group A. The agent to be tested was administered to mice by intraperitoneal injection, and the effect was evaluated with total packed cell volume ratio on the 7th day after tumor implantation. The free fatty acids having 18 carbon atoms were all highly active, but 10-undecenoic acid proved to show no activity. All members of groups B and C were completely ineffective. The sucrose monoelaidate, in particular, and the sucrose monooleate exerted strong effect, whereas other sucrose monoesters exhibited only slight or negative activity. Some basic properties of the two types of carbohydrate esters employed are also described.

Our previous paper reported the results obtained when saturated normal monocarboxylic acids and the ester derivatives were tested for their antitumor activity against Ehrlich ascites carcinoma in mice.[1] In an attempt to provide further informations on the relationship of the chemical structure of the fatty acids and their antitumor activity, our investigation has now been extended to the unsaturated monocarboxylic acids. The agents tested are divided into four groups: A) 10-undecenoic, *trans*-9-octadecenoic (elaidic), *cis*-9-octadecenoic (oleic), *cis*-9, *cis*-12-octadecadienoic (linoleic), and *cis*-9, *cis*-12, *cis*-15-octadecatrienoic (linolenic) acids, B) methyl esters of oleic, linoleic, and linolenic acids, C) 1-O-acyl-β-D-glucopyranose tetraacetates derived from oleic, linoleic, and linolenic acids, and D) so-called "sucrose monoesters" prepared from the acids belonging to group A.

The three compounds mentioned in group C were synthesized according to the same procedures as employed in our previous study for preparation of a series of saturated fatty acyl derivatives of 2,3,4,6-tetra-O-acetyl-β-D-glucopyranose.[4] Although the latter esters were crystallizable, the present unsaturated products were all obtained as viscous semi-solid. Their analytical and spectral data are summarized in Table I. From the results of elemental and gas–liquid chromatographic (GLC) analyses, it was revealed that the preparation from oleic acid was pure enough, but those from linoleic and linolenic acids contained some unidentified impurities (approximate contents; 5 and 7%, respectively). Since the impurities could

1) Part II: Y. Nishikawa, M. Okabe, K. Yoshimoto, G. Kurono, and F. Fukuoka, *Chem. Pharm. Bull.* (Tokyo), 24, 387 (1976).
2) This work was presented partly a) at the 94th Annual Meeting of the Pharmaceutical Society of Japan, Sendai, April, 1974, and partly b) at the 95th Annual Meeting of the Pharmaceutical Society of Japan, Nishinomiya, April, 1975.
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4) Y. Nishikawa, K. Yoshimoto, K. Michishita, and G. Kurono, *Chem. Pharm. Bull.* (Tokyo), 23, 597 (1975).

TABLE I. Analytical and Spectral Data of 1-O-Acyl-β-D-glucopyranose Tetraacetates

Compound Acyl	$[\alpha]_D^{25}$ (c=2.0, $CHCl_3$)	Formula	Analysis (%) Calcd. C	Calcd. H	Found C	Found H	TLC[a] Rf Condition A	TLC[a] Rf Condition B
Oleoyl	+3.5°	$C_{32}H_{52}O_{11}$	62.73	8.55	62.65	8.48	0.44	0.47
Linoleoyl	+4.8°	$C_{32}H_{50}O_{11}$	62.93	8.25	61.70[h]	8.00	0.44	0.28
Linolenoyl	+7.4°	$C_{32}H_{48}O_{11}$	63.14	7.94	62.65[h]	8.48[h]	0.44	0.22
cf.[k] Stearoyl	+4.0°	$C_{32}H_{54}O_{11}$	62.52	8.85	62.65	8.70	0.45	0.59

Compound Acyl	GLC[b] t_R (min) Condition A Main peak	Condition A Minor peak[c]	Condition B Main peak	Condition B Minor peak[c]	IR ν_{max}^{Nujol} cm^{-1} C=O	C=C	β-D-glucosyl	Mass[d] m/e M-347[e]	M+
Oleoyl	8.4	nil[f]	49.6	nil	1760	(1640)[g]	905	265	612
Linoleoyl	8.0[i]	0.7, 1.3, 2.2	50.3	1.3, 1.8, 4.3	1760	1640	905	263	610
Linolenoyl	8.1[j]	0.7, 1.3, 2.2	50.8	1.4, 1.8, 4.4	1760	1640	905	261	608
cf.[k] Stearoyl	9.1	nil	51.9	nil	1750	u.d.[l]	905	267	u.d.

a) condition A: solvent A and untreated Silica gel G
condition B: solvent A and Silica gel G impregnated with $AgNO_3$ (The plates were prepared from the suspension of Silica gel G (30 g) in 12.5% aqueous solution of $AgNO_3$ (60 ml), and, before use, they were activated for 1 hr at 110°.[5])

b) condition A: 1.5% OV-1 on Shimalite W (80—100 mesh); glass column, 1.5 m×4 mm I.D.; carrier gas, N_2 (50 ml/min); column temp., 290°; *cf.* t_R (min) of sucrose octaacetate, 3.0
condition B: 1.5% SE-30 on Chromosorb W (AW-DMCS) (60—80 mesh); glass column, 2.5 m×4 mm I.D.; carrier gas, N_2 (50 ml/min); column temp., 250°; *cf.* t_R (min) of sucrose octaacetate, 23.7

c) peaks attributable to unidentified impurities

d) In general, the fundamental features of the fragmentation patterns of these compounds were similar to those of the corresponding saturated fatty acyl (stearoyl) derivative.[4] But, unlike latter ester, the formers gave weak molecular ion peaks.

e) M-[tetra-O-acetylglucosyloxy]

f) nil: negligible

g) very weak

h) Due to the presence of impurities, satisfactory results were not obtained.

i) peak area ratio (%), *ca.* 95

j) peak area ratio (%), *ca.* 93

k) The data were cited from ref. 4.

l) u.d.: undetectable

not be eliminated by column chromatography, the latter preparations were subjected to the antitumor assay without further purification. Thin-layer chromatographic (TLC) separation of the three esters, which were different in the degree of unsaturation, was achieved satisfactorilly by applicating the Silica gel G plates impregnated with silver nitrate[5]: use of the untreated layer failed to give good resolution. The fundamental features of their infrared and mass spectra were found to show good agreement with those of the saturated analogs reported previously.[4]

The sucrose monoesters used were prepared by the method of Osipow.[6] Each crude product consisted mainly of monoesters, but also contained some di-, tri-, and poly-esters. For removal of the highly substituted minor products, column chromatographic separation was carried out. Thus the fractions which were composed solely of the monoester isomers were isolated as hygroscopic powder. Unlike their parent fatty acids, these ester derivatives were readily soluble in water. The monoester compositions of individual preparations were

5) *a*) C.B. Barrett, M.S.J. Dallas, and F.B. Padley, *J. Am. Oil Chem. Soc.*, **40**, 580 (1963); *b*) H.P. Kaufmann and H. Wessels, *Fette, Seifen, Anstrichmittel*, **66**, 81 (1964).

6) L. Osipow, F.D. Snell, W.C. York, and A. Finchler, *Ind. Eng. Chem.*, **48**, 1459 (1956).

Table II. GLC Analyses of TMS-Derivatives of Sucrose Monoesters[a]

Preparation	Main peak[b] t_R (min)	Minor peaks[c] t_R (min)	Minor peaks[c] Relative area ratio[d]
10-Undecenoate	7.2	6.2	0.11
		8.1	0.18
Elaidate	28.5	25.2	0.16
		32.2	0.12
Oleate	28.4	24.9	0.07
		31.7	0.20
Linoleate	27.4	24.0	0.05
		30.1	0.36
Linolenate[e]	26.3	23.0	0.14
		29.4	0.16

a) conditions: 1.5% OV-1 on Shimalite W (80—100 mesh); 2 m×4 mm I.D. (glass column); N_2, 50 ml/min; column temp., 295° (isothermal)
b) The peak corresponds to peak I shown in Fig. 1 and is probably attributable to the monoester which carries the acyl function at 6-position of the glucose moiety.
c) unidentified peaks corresponding to the peaks II and III shown in Fig. 1
d) relative to the area of the main peak (=1.00)
e) Gas chromatogram is presented in Fig. 1.

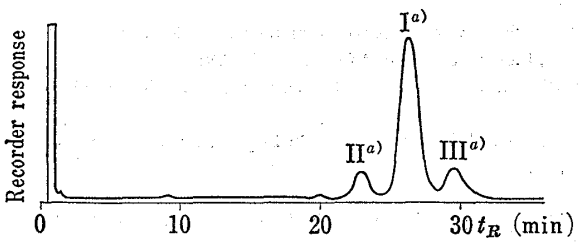

Fig. 1. Gas Chromatogram of Sucrose Monolinolenate as TMS Derivative[b]

a) Peak I, main peak; Peaks II and III, minor peaks (see Table II)
b) 1.5% OV-1 on Shimalite W: operating conditions are shown in Table II.

analysed by GLC. Their chromatograms were mutually similar, each of which consisted of one large peak and two much smaller peaks. The retention times (t_R) and a representative chromatogram are shown in Table II and Fig. 1, respectively. Although conclusive peak identification has not been made as yet, it seems to be probable, by analogy with the preliminarily established composition of the sucrose monolaurate preparation,[1,2b] that the main peak would be attributable to an ester bearing its acyl function at 6-position of the glucose residue.

The antitumor assay was performed under the same conditions as employed in our previous study.[1] The compound to be tested was administered to mice by intraperitoneal injection, and the effect was evaluated with total packed cell volume (TPCV) ratio (% T/C) on the 7th day after tumor implantation.

Experimental

Materials——All the fatty acids and the methyl esters used, except methyl elaidate, were purchased from commercial source (Tokyo Kasei Kogyo Co., Ltd.). These chemicals were of the highest purity available, and used without further purification. Methyl elaidate was prepared by treatment of the parent acid with CH_2N_2 in a usual manner.

Preparation and Properties of 1-O-Acyl-β-D-glucopyranose Tetraacetates——Dry benzene solution (50 ml) of α-acetobromoglucose (2,3,4,6-tetra-O-acetyl-α-D-glucopyranosyl bromide) (10 mmoles) was added dropwise to the suspension of the silver salt of an appropriate fatty acid (12 mmoles) in dry benzene (50 ml), and the reaction mixture was stirred at room temperature for 7 hr. The unreacted silver salt of fatty acid and silver bromide formed were removed by filtration. The filtrate was evaporated to dryness under reduced pressure, and the residue was column chromatographed over Silica gel with a mixture of $(C_2H_5)_2O$: *n*-hexane (2:1) (solvent A) to afford viscous semi-solid. Yields from α-acetobromoglucose, 60—70%. During the storage in a desiccator and under protection from light, the products obtained from linoleic and linolenic acids showed a gradual tendency to become slightly brownish. All the preparations were insoluble in water, but readily soluble in most organic solvents. Their analytical and spectral data are listed in Table I.

Preparation and Properties of Sucrose Monoesters——Well dried sucrose (3 moles) was dissolved in dimethylformamide, and a methyl ester of fatty acid (1 mole) was added with K_2CO_3 (0.1 mole) as an alkaline catalyst. The reaction mixture was maintained at 90—95° under 80—100 mmHg pressure with stirring. After 9 hr, the solvent was distilled off *in vacuo* to give a solid mass, which was washed thoroughly with *n*-hexane for removal of the unreacted methyl ester of fatty acid. The residue was extracted first with acetone and then with *n*-BuOH by heating on a water-bath, and while hot, the undissolved materials (K_2CO_3 and recovered sucrose) were filtered off. Each product, which contained mono-, di-, and poly-esters, was column chromatographed over Silica gel with a mixture of $CHCl_3$: MeOH: AcOH: H_2O (79: 11: 8: 2)[7] (solvent B) to furnish a fraction consisting of monoester isomers as slightly brownish, hygroscopic powder. Yields (g) of the monoester preparations of 10-undecenoic, elaidic, oleic, linoleic, and linolenic acids from 15.40 g of sucrose: 2.65, 2.23, 2.81, 2.48, and 1.98, respectively. When analysed by TLC on Silica gel G layer and with solvent B, each preparation gave one spot. *Rf*-value: monoundecenoate, 0.23; monoelaidate, 0.27; monooleate, 0.27; monolinoleate, 0.29; monolinolenate, 0.30 (Under these conditions, the spots corresponding to di-, tri-, and poly-esters show *Rf*-values higher than those of the monoesters). GLC analyses of the monoester preparations were carried out as their trimethylsilyl (TMS) derivatives obtained by Sweeley's method.[8] Their gas chromatograms were mutually similar, each of which consisted chiefly of one main peak and two minor peaks (Table II, and Fig. 1).

Determination of Antitumor Activity——Ehrlich ascites tumor cells (7×10^6 cells/mouse) were implanted intraperitoneally in ddY mice (female), weighing 23 ± 2 g. The compounds belonging to the groups A, B, and C were insoluble in water; therefore, in these cases, their suspensions in N-saline containing 0.2% Tween 80 were used when they were administered to mice. The sucrose monoester preparations (group D), which were soluble in water, were dissolved in N-saline without containing Tween 80. Treatment was initiated 24 hr after tumor implantation, the agents being given by intraperitoneal injection once daily for 5 consecutive days. On the 7th day after tumor implantation, the antitumor activity, which was expressed as TPCV ratio (%) given by the following equation, and the body weight change (g) were determined. TPCV ratio (%)= (TPCV (ml) of treated group/TPCV (ml) of control group) $\times 100$: where TPCV (ml) = volumes of ascites (ml) $\times$ ascitocrit. The results of the antitumor effect were evaluated as follows:

TPCV ratio (% *T/C*)	0—10	11—40	41—65	66—100
Evaluation	##	#	+	−

Result and Discussion

Table III shows the antitumor activity of all the compounds examined in the present study.

When administered at the standard dose of 400 mg/kg/day $\times$ 5, four of five unsaturated fatty acids tested in the free form, that is, elaidic, oleic, linoleic, and linolenic acids, were found to exert remarkable antitumor activity: 10-undecenoic acid was an only example which showed no activity. Our previous study has revealed that among a series of saturated monocarboxylic acids ranging in carbon chain length from C_3 to C_{18}, lauric (C_{12}) and myristic (C_{14}) acids can exhibit significant activity, while others are almost or completely ineffective.[1] Thus it has proved evident that the mono-, di-, and tri-ethenoic acids possessing 18 carbon atoms are all extremely active, whereas the corresponding saturated fatty acid (stearic) is devoid of the activity. Although so far neither C_{12}-unsaturated acid nor C_{11}-saturated acid has been subjected to the antitumor assay, a striking reverse contrast could also be observed between the activity of 10-undecenoic acid and that of lauric acid, in spite of their close similarity in the carbon chain length. From these findings, it has been suggested that introduction of double bond(s) into the molecule would markedly alter the antitumor effect of the parent compound. In addition, it appears to be probable that the steric nature, *cis* or *trans*, of double bond is an unimportant factor in determining the antitumor activity, since elaidic and oleic acids, a pair of stereoisomers, were both demonstrated to be equally effective.

Using linoleic and linolenic acids, dose response assays were performed. Their antitumor activity tended to be less marked with decreasing dose amounts. Administration at daily

7) The conditions were recommended by Kinoshita for separation of sucrose monoester mixture from di-, tri-, and poly-esters: S. Kinoshita, "Thin-Layer Chromatography, II," (an extra issue (No. 64) of the *Kagaku No Ryoiki*), ed. by S. Hara, O. Tanaka, and S. Takitani, Nankodo Co. Ltd., Tokyo, 1964, p. 79.

8) C.C. Sweeley, R. Bentley, M. Makita, and W.W. Wells, *J. Am. Chem. Soc.*, **85**, 2497 (1963).

Table III. Antitumor Activity of Unsaturated Fatty Acids and the Ester Derivatives (TPCV Method[a])

Compound	Dose (mg/kg/day)	TPCV ratio[b] (% T/C)	Evaluation of Activity	Body wt. change (g)[b] Treated	Body wt. change (g)[b] Control	Deaths/Total Treated	Deaths/Total Control
A) Free acid							
10-Undecenoic acid	400×5	87.7	—	−0.4	+0.2	1/6	1/6
Elaidic acid	400×5	6.7	##	+1.2	+0.2	0/6	1/6
Oleic acid	400×5	0.0	##	−0.1	+1.3	0/6	0/6
	400×5	8.4	##	−0.01	+0.8	0/6	0/6
Linoleic acid	400×5	0.0	##	−1.5	−0.1	0/6	1/6
	400×5	7.1	##	+0.2	+0.06	0/6	1/6
	300×5	17.2	#	−0.03	+0.06	0/6	1/6
	200×5	17.2	#	−0.7	+0.06	0/6	1/6
	100×5	69.2	—	−2.7	+0.06	3/6	1/6
Linolenic acid	400×5	0.0	##	+0.1	+0.9	0/6	0/6
	400×5	13.2	#	−0.5	−2.0	0/6	1/6
	300×5	27.3	#	−1.3	−2.0	1/6	1/6
	200×5	95.0	—	+0.7	−2.0	1/6	1/6
	100×5	138.0	—	−1.3	−2.0	0/6	1/6
cf.[c] Lauric acid	400×5	0.5	##	−3.1	+0.1	0/6	0/5
cf.[c] Stearic acid	400×5	90.0	—	−3.1	−1.3	1/6	0/6
B) Methyl ester							
Oleate	400×5	84.5	—	−2.3	−2.6	2/6	0/6
Linoleate	400×5	125.5	—	−2.1	−0.1	1/6	1/6
Linolenate	400×5	96.4	—	−3.4	+0.9	1/6	0/6
C) 1-O-Acyl-β-D-glucopyranose tetraacetate							
Oleate	400×5	104.9	—	−1.2	−2.6	2/6	0/6
Linoleate	400×5	158.2	—	−1.8	−0.1	0/6	1/6
Linolenate	400×5	123.7	—	−0.6	+0.9	0/6	0/6
D) Sucrose monoester							
10-Undecenoate	250×5	68.7	—	−0.8	+1.0	0/6	0/6
Elaidate	250×5	1.2	##	+0.7	+1.0	1/6	0/6
Oleate	250×5	13.5	#	+0.2	+1.0	0/6	0/6
Linoleate	250×5	61.6	+	−0.2	−0.2	0/6	0/6
Linolenate	250×5	50.6	+	−0.4	−0.2	0/6	0/6

a) tumor, Ehrlich ascites carcimoma intraperitoneally implanted in ddY mice: route, intraperitoneal injection
b) determined on the 7th day after tumor implantation
c) Data were cited from ref. 1.

dose of 300 mg/kg diminished, though slightly, the activity of both acids. In the case of linoleic acid, daily dose of 200 mg/kg was still effective. Reduction of daily dose to 100 mg/kg resulted in complete loss of the antitumor effect of both acids. Some saturated fatty acids have previously been reported to show similar tendency.[1)]

The strong antitumor effect of oleic, linoleic, and linolenic acids was completely prevented by methyl substitution. All the 1-O-acyl-β-D-glucopyranose tetraacetates derived from these fatty acids were also found to be inactive. Both the results are, however, not surprising, since we have already recognized in our preceeding study that esterification of saturated fatty acids by a methyl group can significantly alter—improve or reduce—the antitumor activity of the parent compounds, and that this type of carbohydrate esters prepared from eleven saturated fatty acids are ineffective without exception.[1)]

On the other hand, the sucrose monoester preparation of elaidic acid was shown to exert the pronounced antitumor activity, even when it was administered at the dose of 250 mg/kg/day ×5 (The fatty acid content contained in 250 mg of the preparation is calculated to be *ca.* 110 mg). Considerable effectiveness was also exhibited with the sucrose monooleate. The sucrose monoesters of linoleic and linolenic acids were, however, almost inactive, although the

antitumor effect of their parent fatty acids was as remarkable as that of elaidic or oleic acid. The monoundecenoate whose parent fatty acid was ineffective showed no activity. As reported previously, the sucrose monoesters of some saturated fatty acids, such as caprylic, lauric, and myristic acids, have been suggested to be very active.[1] But they showed a tendencey to evoke strong toxicity. Thus it seems to be noteworthy that all the sucrose monoesters used in the present study were apparently non-toxic. By GLC analyses, each sucrose monoester preparation was demonstrated to consist of a complex mixture of several positional isomers (Table II, and Fig. 1). In an effort to determine the monoester compositions of these preparations, further chemical investigations are now in progress.

So far relatively few references to the antitumor properties of unsaturated fatty acids and related compounds have appeared. The first paper in this field was published by Nakahara in 1922.[9] He has revealed that unsaturated fatty acids such as oleic, linoleic, and linolenic acids had a distinct immunizing action against transplantable cancer in mice, and that this action was not shared by saturated fatty acids. Similar observation was also reported by Bierich.[10] Townsend, *et al.* found that 10-hydroxy-2-decenoic acid from royal jelly suppressed the development of a transplantable mouse leukemia and the formation of ascitic tumors in mice.[11] Successively, they indicated that most of the mono- and di-carboxylic acids, when mixed with three different ascites tumor cells (Ehrlich carcinoma, 6C3HED lymphosarcoma, and TA_3 mammarycarcinoma) at pH values below 5.0 and prior to inoculation into mice, completely suppressed the development of the ascitic tumor, but when mixed at physiological pH values, only 2-decenoic, linoleic, and linolenic acids showed the activity. Yamamoto and co-workers demonstrated that the unsaturated fatty acid fraction (main constituents; oleic and linolenic acids) obtained from the X-ray irradiated rabbit showed *in vivo* antitumor activity against the Brown-Pearce sarcoma of rabbit, Ehrlich tumor in solid form, and several human carcinomas.[12] Sodium oleate,[13] and oxidized linolenic acid and its methyl ester[14] have been mentioned to possess activity upon the Rous sarcoma, Bennet reported that oleic acid strongly depressed oxygen uptake of Gardner lymphosarcoma, and Ehrlich ascites tumor cells, and that tumor cell membranes acted upon by this acid were damaged in a manner similar to the process of hemolysis.[15]

Recently, Arima, *et al.* have found in their screening for antitumor antibiotics that a fatty acid fraction consisting mainly of oleic and linoleic acids and the corresponding monoglyceride fraction, both of which were extracted from some fungal mycelia, exerted significant activity against Ehrlich ascites carcinoma by intraperitoneal administration.[16a–e] The major criterion

9) *a*) W. Nakahara, *J. Exptl. Med.*, **35**, 493 (1922); *b*) *Idem*, *ibid.*, **40**, 363 (1924); *c*) *Idem*, *ibid.*, **41**, 347 (1925); *d*) *Idem*, *Gann*, **19**, 1 (1925).

10) R. Bierich, *Leeuwenhoek-Vereeniging*, **1**, 14 (1922).

11) *a*) G.F. Townsend, J.F. Morgan, S. Tolnai, B. Hazlett, H.J. Morton, and R.W. Shuel, *Cancer Res.*, **20**, 503 (1960); *b*) G.F. Townsend, W.H. Brown, E.E. Felauer, and B. Hazlett, *Canad. J. Biochem. Physiol.*, **39**, 1765 (1961); *c*) S. Tolnai and J.F. Morgan, *ibid.*, **40**, 869, 1367 (1962); *d*) *Idem*, *ibid.*, **44**, 979 (1966).

12) *a*) M. Yamamoto, K. Utsumi, and S. Seno, *Acta Med. Okayama*, **17**, 129 (1963); *b*) T. Ofuji, *ibid.*, **18**, 55 (1964); *c*) S. Seno, and M. Yamamoto, *ibid.*, **19**. 59 (1965); *d*) M. Yamamoto, T. Shiwaku, K. Ando, T. Tanabe, N. Katsumata, and Y. Hada, *Okayama Igaku Zasshi*, **75**, 695 (1963).

13) A.M. Begg and H.A.A. Aitken, *Brit. J. Exptl. Pathol.*, **13**, 479 (1932).

14) B. Sokoloff, M. Toyomizu, C.C. Saelhof, B. McConnell, and F. Zbar, *Growth*, **22**, 215 (1958).

15) L.R. Bennet and F.E. Connon, *J. Natl. Cancer Inst.*, **19**, 999 (1957).

16) *a*) G. Tamura, A. Kato, K. Ando, K. Kodama, S. Suzuki, K. Suzuki, and K. Arima, *J. Antibiotics*, **21**, 688 (1968); *b*) K. Ando, S. Suzuki, K. Suzuki, K. Kodama, A. Kato, G. Tamura, and K. Arima, *ibid.*, **21**, 690 (1968); *c*) K. Ando, A. Kato, G. Tamura, and K. Arima, *ibid.*, **22**, 23 (1969); *d*) A. Kato, K. Ando, K. Kodama, G. Tamura, and K. Arima, *ibid.*, **22**, 77 (1969); *e*) K. Ando, A. Kato, S. Suzuki, G. Tamura, and K. Arima, "Progress in Antimicrobial and Anticancer Chemotherapy, Proceedings of the 6th International Congress of Chemotherapy," Vol. II, ed. by H. Umezawa, University of Tokyo Press, Tokyo, 1970, p. 136; *f*) A. Kato, K. Ando, S. Suzuki, G. Tamura, and K. Arima, *ibid.*, p. 142; *g*) A. Kato, K. Ando, G. Tamura, and K. Arima, *Cancer Res.*, **31**, 501 (1971): *h*) K. Ando, K. Kodama, A. Kato, G. Tamura, and K. Arima, *ibid.*, **32**, 125 (1972).

employed in their experiments was survival. Subsequently they have revealed that the antitumor activity is a common property of some higher fatty acids and related derivatives.[16e–h] Among the unsaturated compounds examined by them, linoleic, linolenic, elaidic, vaccenic, and elucic acids, and sucrose esters of oleic, elaidic, and linoleic acids have been manifested to be highly effective in inhibiting tumor growth and elongating the life span[16e,f]: oleic acid and methyl esters of oleic and linoleic acids were inferior to above agents. They also indicated that an artificial mixture of oleic and linoleic acids in a ratio of 1:1 inhibited the growth of Ehrlich solid tumor, when it was administered subcutaneously.[16e] In general, there appears to be good agreement between their results and our present findings.

In our present and previous studies, it has been confirmed that some fatty acids, saturated and unsaturated, as well as some ester derivatives can exert marked antitumor activity against Ehrlich ascites carcinoma by intraperitoneal administration. The effects of these compounds against other tumors and through other injection routes are now being tested. At present, no reasonable interpretation of the structure-activity relationships observed through our experiments is available. The exact mechanism involved in the antitumor activity of fatty acids is also still unknown, though some suggestions have been proposed by Arima, *et al.*[16f,g] These problems remain to be solved in future.

Acknowledgement The authors express their deep gratitude to Dr. W. Nakahara, President of the National Cancer Center, for his kind advices and suggestions, and to Dr. G. Kurono, Emeritus Professor of Kanazawa University, for his encouragement. Thanks are also due to Miss M. Okada for her skillful technical assistance. This work was supported in part by a Grant-in-Aid from the Ministry of Health and Welfare, which is greatfully acknowledged.