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Chemical and Biochemical Studies on Carbohydrate Esters. IX.[1] Antitumor Effects of Selectively Fatty Acylated Products of Maltose[2]

Yoshihiro Nishikawa,*[,3a] Kimihiro Yoshimoto,[3a] Munekazu Nishijima,[3a] Fumiko Fukuoka,[3b] and Tetsuro Ikekawa[3b]

Faculty of Pharmaceutical Sciences, Kanazawa University[3a] 13-1 Takara-machi, Kanazawa 920, Japan, and National Cancer Center Research Institute,[3b] 5-1-1 Tsukiji, Chuo-ku, Tokyo 104, Japan

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Selective fatty acylation of maltose was carried out by heating an equimolar mixture of the disaccharide and an appropriate acid chloride in pyridine. The resulting crude product was chromatographed to furnish two preparations, termed maltose-[mono]- and -[high]-esters; the former preparation was an isomeric mixture of monoesters among which the 1-, 6-, and 6′-isomers were presumably predominant, and the latter consisted of di-, tri-, and poly-esters, as indicated by gas-liquid and thin-layer chromatographies. The antitumor effects of the maltose-[mono]-esters of stearic, palmitic, myristic, lauric, and caprylic acids towards Ehrlich ascites carcinoma in mice were tested by the total packed cell volume method, by intraperitoneal injection. The stearic, palmitic, and myristic esters proved to be effective, while the lauric and caprylic esters exhibited a slight effect and no effect, respectively. The antitumor effect of the maltose-[mono]-stearate upon the same tumor was compared with that of the analogous derivative of sucrose by survival bioassay (intraperitoneal administration) at various doses. Both compounds showed similar antitumor results, giving especially marked effects at the dose of 50 mg/kg/day × 5. These two compounds also exerted moderate growth-inhibitory effects against solid sarcoma 180 in mice when they were administered subcutaneously; in the case of sucrose-[mono]-stearate, intramuscular injection was also effective. The maltose-[high]-esters of stearic, palmitic, and myristic acids failed to exert marked activity upon this solid tumor by either administration route.

Keywords——fatty acylation of maltose; maltose esters of fatty acids; sucrose monoester of stearic acid; gas-liquid chromatography; thin-layer chromatography; antitumor effect; Ehrlich ascites carcinoma; solid sarcoma 180; total packed cell volume method; survival bioassay

In previous studies of this series,[4] sucrose- and trehalose-esters of various long-chain fatty acids have been examined for antitumor activities, and some of the samples tested proved to possess *in vivo* and *in vitro* antitumor effects against certain tumors. As a continuation of this research, fatty acid esters of maltose (4-O-α-D-glucopyranosyl-D-glucopyranose) (**1**) have now been subjected to *in vivo* antitumor bioassays in an attempt to compare the antitumor activities of the esters of reducing and non-reducing disaccharides.

Test Samples

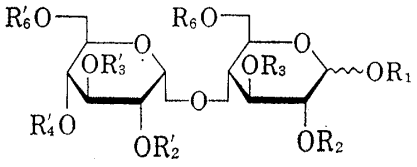
So far little work has been done on the selective fatty acylation of maltose, in spite of extensive investigations on its selective benzoylation.[5] The sucrose- and trehalose-esters used in our previous antitumor tests were prepared by transesterification of the disaccharide with a methyl ester of a fatty acid in the presence of an alkaline catalyst. However, according to Linfield *et al.*, the successful fatty acylation of lactose could be achieved by the acid chloride method, but not by the transesterification process.[6] Therefore, the acid chloride method was adopted for the production of the maltose-esters.

The general procedure employed for preparation of the test sample is shown in Chart 1. An equimolar mixture of anhydrous maltose and an appropriate fatty acid chloride, such as capryloyl, lauroyl, myristoyl, palmitoyl, or stearoyl chloride, was heated at 95—100° for 15 hr

an equimolar mixture of anhydrous maltose (**1**) and an acid chloride
—stirring in pyridine at 90—100° for 15 hr
—evaporation to dryness *in vacuo*
residue
—washing with *n*-hexane
—extraction with hot acetone
extract
—evaporation to dryness *in vacuo*
crude product
—column chromatography on silica gel with $CHCl_3$–MeOH (9:1)

maltose-[mono]-ester
major components;
1-(**2**), 6′-(**3**), and 6-monoester (**4**)
minor components;
positional isomers otehr than 3- and 3′-monoesters (**5** and **6**)

maltose-[high]-ester
major components;
diester (s)
minor components;
tri- and polyesters



Compd. No.	R_1	R_2	R_3	R_6	R_2'	R_3'	R_4'	R_6'
1	H	H	H	H	H	H	H	H
2	F	H	H	H	H	H	H	H
3	H	H	H	H	H	H	H	F
4	H	H	H	F	H	H	H	H
5	H	H	F	H	H	H	H	H
6	H	H	H	H	H	F	H	H
cf. MTP (**7** or **8**)								
7	P	H	H	P	P	H	H	P
8	P	H	H	P	H	H	P	P

F: capryloyl, lauroyl, myristoyl, palmitoyl, or stearoyl.
P: palmitoyl.

Chart 1. General Preparation Procedure and Chemical Composition of Test Sample

in pyridine to afford a crude product. The composition of the resulting product was analyzed by thin-layer chromatography (TLC) on silica gel, with chloroform–methanol–acetic acid–water (79: 11: 8: 2 by volume) (solvent A) as a developing solvent. The TLC conditions are known to be suitable for the resolution of a mixture containing mono-, di-, tri-, and polyesters of sucrose.[4b-d,7] Each chromatogram obtained consisted of many (about ten) spots, among which the slowest-moving and the second slowest-moving spots were relatively larger than other faster-moving spots, and were termed spots A and B, respectively: introduction

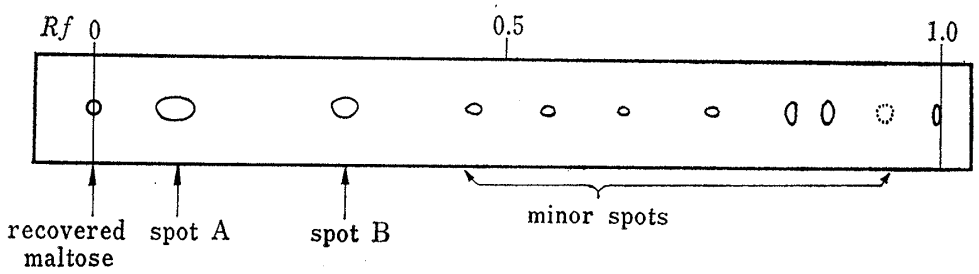


Fig. 1. TLC of the Crude Product obtained by Selective Lauroylation of Maltose
Conditions: on silica gel with solvent A.

of a longer fatty acyl function tended to give spots with higher mobilities. A representative chromatogram is illustrated in Fig. 1.

A small portion of the lauroylated product was applied to a silica gel column and eluted with a mixed solvent of chloroform–methanol (9: 1) to furnish two fractions corresponding to the spots A and B. In order to determine the acyl contents of these two fractions, their methanolysates were analyzed by gas-liquid chromatography (GLC). Based on the results obtained, the major spots A and B were assigned to mono- and di-substituted esters, respectively, and, hence, the minor spots were considered to be ascribable to polyester components.

Each crude product was then chromatographed on a preparative scale under the conditions described above to yield two preparations designated as maltose-[mono]- and -[high]-esters, respectively: the former preparation consisted of the monoesters corresponding to spot A, while the latter contained di- and poly-esters corresponding to spot B and minor spots.

As reported already, satisfactory resolution of an isomeric mixture of sucrose-monoesters could be attained by TLC on silica gel, with chloroform–methanol (82: 11, v/v) (solvent B) as a developing solvent; the use of solvent A resulted in an apparently single spot.[4b,8] The maltose-[mono]-esters failed to give multiple spots, even when solvent B was employed. They were not homogeneous, however, as evidenced by GLC, which was carried out after formation of the trimethylsilyl (TMS) derivatives, on OV-1 and OV-17 columns. Five chromatograms obtained on either column showed similarity in their peak patterns. Representative chromatograms are depicted in Fig. 2. In the case of the OV-1 column, chromatograms each

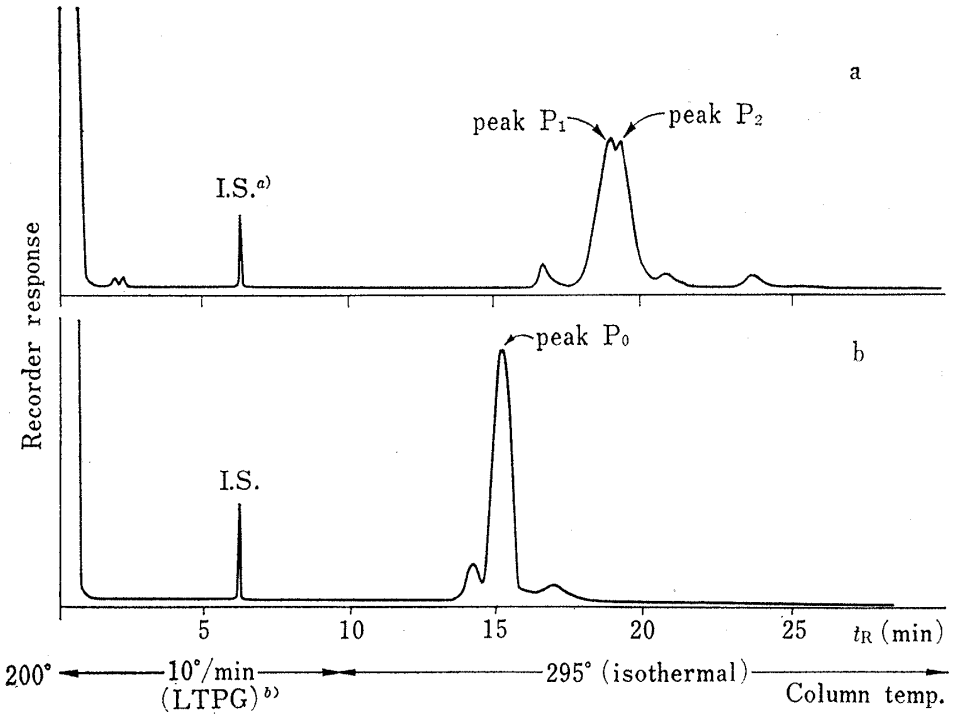


Fig. 2. GLC of Maltose-[mono]-laurate as the TMS Derivative[c)]

a) I.S.: internal standard; phenyl β-D-glucopyranoside as the TMS derivative.
b) LTPG: linear temperature-programmed operation.
c) GLC Conditions:

Fig. No.	Column packing	Column size	Flow rate of carrier gas(N_2)
a	1.5% OV-17 on Shimalite W (80—100 mesh)	2.5 m×4 mm (I.D.)	50 ml/min
b	1.5% OV-1 on Shimalite W (80—100 mesh)	2.0 m×4 mm (I.D.)	50 ml/min

Table I. Retention Times of the Major Peaks in Gas Chromatograms of the TMS derivatives of Maltose-[mono]-esters[a,b]

Sample	Peak P_1		Peak P_2	
	t_R(min)	rt_R[c]	t_R(min)	rt_R[c]
Maltose-[mono]-caprylate	5.0	3.86	5.2	4.00
Maltose-[mono]-laurate	12.2	9.42	12.5	9.58
Maltose-[mono]-myristate	17.2	13.21	17.6	13.53
Maltose-[mono]-palmitate	25.2	19.36	25.7	19.78
Maltose-[mono]-stearate	33.0	25.38	33.8	26.02

a) GLC conditions: column packing, 1.5% OV-17 on Shimalite W (80—100 mesh); column size, 2.0 m×4 mm I.D.; column temp., 295° ((isothermal); carrier gas, N_2 (50 ml/min).
b) *cf.* Figs. 2 and 3.
c) Internal standard: TMS-maltose; t_R, 1.3 min and rt_R, 1.00.

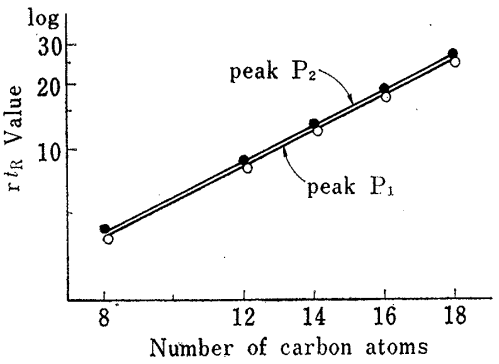


Fig. 3. Relationships between the rt_R Values of Peaks P_1 and P_2 and the Carbon Numbers of Acyl Functions Contained in Maltose-[mono]-esters[a]

a) GLC conditions used were the same as in Table I.

consisting of one large peak (peak P_0) and a few much smaller peaks were obtained. Use of the OV-17 column resulted in better resolution, indicating that the major peak was further separable into two peaks (peaks P_1 and P_2) with approximately equal intensities. The retention times (t_R) and the relative t_R (rt_R) of the peaks P_1 and P_2 are listed in Table I. It is of interest that linear relationships could be observed between the log rt_R values and the carbon numbers of the acyl functions introduced, as shown in Fig. 3: such relationships had been established before with the D-glucopyranosyl monoester isomers.[9] Our preliminary examination suggested that peak P_1 could be attributed to the 1-monoester (**2**) and peak P_2 to the 6′- and 6-isomers (**3** and **4**).[2a,10] The components responsible for the minor peaks have not been identified as yet, though it is almost certain that 3- and 3′-isomes (**5** and **6**) were absent in the maltose-[mono]-esters.[2a,10] However, in connection with the composition of the selectively fatty-acylated product of maltose, it may be worth mentioning that, based on studies by various workers, the order of reactivity of eight hydroxyl groups in this disaccharide towards benzoylation appears to be as follows[5]: HO-1, HO-6′>HO-2, HO-6>HO-2′≈HO-3′>HO-4′>HO-3.

Besides the maltose esters, sucrose-[mono]-stearate was used as a test sample in most of the present bioassays. This preparation (main component, the 6-monoester) was obtained by transesterification, followed by column chromatographic fractionation of the crude product, as described in our preceding papers.[4b,d]

Antitumor Effects

Throughout the present bioassays, six mice were used for each test sample, and, unless otherwise stated, physiological saline was employed as a vehicle.

First, the antitumor effects of the five maltose-[mono]-esters upon Ehrlich ascites carcinoma were examined by the total packed cell volume (TPCV) method.[11] The tumor cells were implanted intraperitoneally (*i.p.*) into mice and the sample was administered *i.p.* at a dose of 250 mg/kg for four or five consecutive days, starting 24 hr after the tumor inoculation. The activity was evaluated on the seventh day after the tumor implantation. As can be seen in Table II, the maltose-[mono]-esters of stearic and palmitic acids in particular, as well

Table II. Antitumor Effects of Maltose-[mono]-esters upon Ehrlich Ascites Carcinoma (TPCV method)

Sample	Dose (mg/kg × days)	TPCV ratio (%, treated/control)	Evaluation of activity[a)]	Body wt. change(g)	
				Treated	Control
Maltose-[mono]-caprylate	250×5	71	—	+1.2	+2.7
Maltose-[mono]-laurate	250×4	47	+	+0.3	+2.0
Maltose-[mono]-myristate	250×5	13	++	−0.4	+2.0
Maltose-[mono]-palmitate	250×4	1	+++	−0.8	+2.0
Maltose-[mono]-palmitate	50×5	12	++	+0.9	+0.4
Maltose-[mono]-stearate	250×4	0	+++	+1.1	+2.0

a) Criteria:

TPCV ratio (% treated /control)	0—10	11—40	41—65	>65
Evaluation of activity	+++	++	+	−

as of myristic acid, were highly effective, while those of lauric and caprylic acids exerted only a slight effect and no effect, respectively.

Analogous monoester derivatives of sucrose and trehalose have already been tested under similar bioassay conditions.[1,4a,b,d)] According to the results, the stearoyl, palmitoyl, and myristoyl monoesters of these non-reducing disaccharides were markedly effective and their lauroyl and capryloyl analogs tended to exhibit lower activities. In addition, it was observed with some of the previous samples involving sucrose-[mono]-palmitate that a decrease in the dose to 50 mg/kg/day×5 caused little or no loss of the antitumor effect.[4b)] In the case of maltose-[mono]-palmitate, the effectiveness of the dose of 50 mg/kg/day×5 has now been confirmed.

On the basis of our present and earlier findings, it became evident that, as far as this bioassay system is concerned, the antitumor behavior of the disaccharide monoester would not be altered significantly by replacing the non-reducing carbohydrate unit with the reducing one.

Secondly, the antitumor effect of maltose-[mono]-stearate upon the same carcinoma was evaluated by the survival method, in comparison with that of sucrose-[mono]-stearate. The test sample was administered *i.p.* to the mice bearing *i.p.* implanted tumor cells once daily for five consecutive days, starting 24 hr after the tumor transplantation. The mean survival time (MST) and increase in life-span (ILS) obtained are shown in Table III. In Experiment i), both samples were tested at daily doses of 250, 100, and 50 mg/kg. The survival effects of sucrose-[mono]-stearate at the first two doses have been reported already; 100 mg/kg was indicated to be more effective than 250 mg/kg.[1)] The present data for this sample confirmed the above results; in addition, it was found that the newly employed dose, that is, 50 mg/kg, was most effective. The antitumor effects of maltose-[mono]-stearate at these three

Table III. Antitumor Effects of Maltose- and Sucrose-[mono]-stearates upon Ehrlich Ascites Carcinoma (Survival Method)

Exp. No.	Dose (mg/kg × days)	Maltose-[mono]-stearate		Sucrose-[mono]-stearate	
		MST(days)	ILS(%)	MST(days)	ILS(%)
Experiment i)	250×5	17.2	54	22.7	96
	100×5	29.3	162	35.7	208
	50×5	42.3	278	38.0	228
	Control	11.2		11.6	
Experiment ii)	30×5	32.5	126	31.7	120
	10×5	21.3	48	16.8	17
	3×5	16.8	17	16.8	17
	Control	14.4		14.4	

doses proved to be grossly comparable with those of sucrose-[mono]-stearate. In Experiment ii), daily doses of 30, 10, and 3 mg/kg were adopted. It was observed with either sample that the daily dose of 30 mg/kg could produce a relatively high ILS value, but it was much less effective than 50 mg/kg, and the two other doses failed to give marked effects. Accordingly, the optimum dose for both samples has now been concluded to be 50 mg/kg/day×5: the reason why the larger doses are less effective is unclear, but may be related to the increasing toxicity. On the basis of these findings, it appeared probable that the antitumor activity of the reducing disaccharide monoester would be similar to that of the analogous derivative of non-reducing disaccharide again in this bioassay system.

Thirdly, antitumor tests against solid sarcoma 180 were conducted with the maltose- and the sucrose-[mono]-stearates. Our preceding studies have shown that, when injected *i.p.*, the latter compound failed to inhibit the growth of this solid tumor, though it was somewhat effective against the same tumor in ascites form as judged by the TPCV and survival methods.[4a] In the present bioassays, each test sample was administered subcutaneously (*s.c.*). The tumor cells were implanted *s.c.* into the right groin of mice, and starting from the next day, test sample was injected *s.c.* in the back every other day until the tenth administration. The antitumor effect was evaluated at the end of the fourth week by measuring the tumor weight. As shown in Table IV, moderate antitumor effects were seen with both samples at the daily dose of 30 mg/kg: in the case of sucrose-[mono]-stearate, the dose of 3 mg/kg/day was also effective. Complete regression of the tumor, however, was not observed in any of the mice treated with either sample. The results suggest that carbohydrate monoesters of this type possess antitumor activity not only against ascites tumors but also against certain solid tumors.

Table IV. Antitumor Effects of Maltose- and Sucrose-[mono]-stearates upon Solid Sarcoma 180

Sample	Dose[a] (mg/kg × days)	Inhibition ratio (%)	Evaluation of activity[b]	Body wt. change(g)
Maltose-[mono]-stearate	30×10	61.0	##	+3.9
Sucrose-[mono]-stearate	30×10	53.9	##	+5.3
	3×10	53.7	##	+4.4
Control				+2.6

a) Administration route, *s.c.*
b) Criteria:

Inhibition ratio (%)	0—10	11—30	31—50	51—70	71—100
Evaluation of activity	−	±	+	##	##

During the course of our present work, a synthetic maltose-tetrapalmitate (MTP), which was identified tentatively as 1,6,2′,6′- or 1,6,4′,6′-tetra-O-palmitoyl maltose (**7** or **8**), was reported by Nigam *et al.* to be a nontoxic immunopotentiator with antitumor activity.[12] According to their observation, the degree of sensitivity to MTP varies with the tumor-host system: two solid tumors, L26 and B16, transplanted *s.c.* in mice were highly sensitive to *s.c.* MTP injection. Thus, it seemed worth testing the antitumor abilities of maltose-[high]-esters against the solid sarcoma 180.

Fourthly, the antitumor effects of the maltose-[high]-esters of stearic, palmitic, and myristic acids were compared with that of sucrose-[mono]-stearate under the conditions described for the third bioassay: owing to the shortage of material, maltose-[mono]-stearate was not used here. The results obtained are shown in Table V. When injected *s.c.* at daily doses of 30 and 10 mg/kg, the three maltose-[high]-esters were nearly or completely ineffective, whereas sucrose-[mono]-stearate showed moderate activities. In addition, as can be seen in Table V, intramuscular (*i.m.*) administration of these samples gave results similar to those obtained by *s.c.* injections.

Table V. Antitumor Effects of Maltose-[high]-esters and Sucrose-[mono]-stearate upon Solid Sarcoma 180

Sample	Dose (mg/kg × days)	*s.c.* injection		*i.m.* injection	
		Inhibition ratio (%)	Evaluation of activity[a)]	Inhibition ratio (%)	Evaluation of activity[a)]
Maltose-[high]-myristate	30×10	42.7	+	37.1	+
	10×10	11.5	±	1.4	−
Maltose-[high]-palmitate	30×10	34.4	+	7.1	−
	10×10	11.5	±	30.0	±
Maltose-[high]-stearate	30×10	16.4	±	−7.1	−
	10×10	4.9	−	38.6	+
Sucrose-[mono]-stearate	30×10	57.4	++	52.9	++
	10×10	62.3	++	55.8	++

a) For criteria, see Table IV.

Thus, taking the aforementioned data for maltose-[mono]-stearate into account, we consider that, in this bioassay system, monoesters produce more promising effects than the highly substituted analogs. The same tendency had been pointed out previously with the sucrose esters, when their *in vivo* and *in vitro* antitumor activities were examined against Ehrlich ascites carcinoma and mouse leukemia L-5178Y, respectively.[1,4a,b)] As described above, compositional analysis of the maltose-[high]-esters has not been completed, and hence it is not yet clear whether the maltose-[high]-palmitate used contained a tetraester corresponding to MTP. Accordingly, at this time, it cannot be conclusively ruled out that the inactivity of the maltose-[high]-palmitate is due to the absence, or inadequate content, of MTP. However, it appears to be more probable that the apparent discrepancy observed between the antitumor activities of these two samples can be accounted for by the difference in the tumor-host systems employed.

Experimental

General——TLC was performed on Silica Gel G (Merck) and detection of the spots was effected with 10% H_2SO_4 followed by heating. GLC was carried out with a Shimadzu gas chromatograph, model GC-4BPF, equipped with a hydrogen flame ionization detector and a glass column. Column chromatography was conducted on silica gel (Wakogel 200), using a mixed solvent of $CHCl_3$–MeOH (9: 1 v/v) for elution. Throughout the present bioassays, six mice were used for each test sample, and unless otherwise stated, physiological saline was employed as a vehicle.

Preparation of Test Sample——(i) Maltose-[mono]- and -[high]-esters: The general procedure used is shown in Chart 1. Anhydrous maltose (**1**) was obtained by dissolving the commercial dihydrate in pyridine and distilling the solution at atmospheric pressure in order to achieve azeotropic removal of the water of crystallization.[13)] An equimolar mixture of anhydrous maltose and an appropriate fatty acid chloride, such as stearoyl, palmitoyl, myristoyl, lauroyl, or capryloyl chloride, (45 mmol each) in pyridine (150 ml) was heated at 95—100° with stirring. After 15 hr, the solution was evaporated to dryness under reduced pressure and the residue was washed with *n*-hexane to remove the unreacted acid chloride. The resultant material was extracted twice with hot acetone (200 ml × 2), and the extract was evaporated to dryness *in vacuo* to afford a crude product as a slightly brownish, hygroscopic solid; yield, 10—12 g. The five crude products obtained were analyzed by TLC, using solvent A (see above). Each chromatogram consisted of many spots, among which the slowest-moving and the second slowest-moving spots were relatively larger than other, faster-moving spots, and were termed spots A and B, respectively. The *Rf* values of the spots A were around 0.15 and those of the spots B of the capryloyl, lauroyl, myristoyl, palmitoyl, and stearoyl products were 0.21, 0.30, 0.35, 0.40, and 0.45, respectively. A representative chromatogram is shown in Fig. 1. A small portion of the lauroylated product was subjected to column chromatography, and two preparations corresponding to spots A and B were isolated. Their acyl contents were determined to be 35.4% and 52.9%, respectively, by GLC (5% DEGS, 150°) analysis of the methanolysates (2.5% CH_3OH–HCl, 100°, 2 hr); the acyl contents calculated for the mono- and dilaurates of maltose are 34.9% and 51.9%, respectively. Each crude product was then column chromatographed on a preparative scale. The faster-eluted fractions corresponding to the minor spots and spot B were combined together to furnish the preparation termed maltose-[high]-ester.

Subsequent elution afforded the maltose-[mono]-ester, which gave only spot A on TLC. The ratio of maltose-[mono]-ester to maltose-[high]-ester was approximately 2:5 (by weight). All preparations were obtained as slightly hygroscopic, colorless powders, and their infrared spectra (KBr) showed ester bands at around 1730 cm^{-1}. The maltose-[mono]-esters were soluble in H_2O, MeOH, EtOH, AcOH, pyridine, and N,N-dimethylformamide, and, when warmed, in $CHCl_3$ and acetone, but were insoluble in $(C_2H_5)O$, CCl_4, and benzene. The maltose-[high]-esters were sparingly soluble in H_2O, but soluble in $CHCl_3$, benzene, MeOH, EtOH, and pyridine, and, when warmed, in AcOEt and acetone. $[\alpha]_D^{17}$ ($c=2$, MeOH) values of the maltose-[mono]-esters: caprylate, +60.3° (+65.1°); laurate, +63.9° (+67.4°); myristate, +60.1° (+63.7°); palmitate, +62.3° (+68.6°); stearate, +67.5° (+69.9°) (the figures in parentheses show the values obtained after standing for 24 hr). On TLC with triple development with solvent B (see above), the maltose-[mono]-esters of caprylic, lauric, myristic, palmitic, and stearic acids gave single spots with the following *Rf* values; 0.28, 0.35, 0.38, 0.41, and 0.45, respectively (*cf.* phenyl β-D-glucopyranoside, 0.56). The maltose-[mono]-esters were converted into the TMS derivatives according to the procedure of Sweeley,[14] and analyzed by GLC on OV-1 and OV-17 columns. The features of the chromatograms obtained have already been described. Representative peak patterns and the t_R and rt_R values of the major peaks are shown in Fig. 2 and Table I, respectively, together with the operating conditions employed. The linear relationships between the rt_R values and the carbon numbers of the acyl functions are depicted in Fig. 3.

(ii) Sucrose-[mono]-stearate: This preparation was obtained by transesterification, followed by column chromatographic fractionation of the crude product as described in our preceding paper.[4b] By TLC and GLC analyses, the composition of this sample was confirmed to be identical with that of the previous specimen reported in ref. 4*b*) under the name of preparation C (or C').

Bioassay Methods——(i) Activity Assay of Maltose-[mono]-esters upon Ehrlich Ascites Carcinoma by the TPCV Method: The general bioassay conditions were similar to those adopted in our previous studies.[1,4] Seven-day-old Ehrlich ascites carcinoma (about 10^7 cells/mouse) was inoculated *i.p.* into female ddY strain mice weighing 22 ± 2 g. The test sample was injected *i.p.* once daily for four or five consecutive days, starting 24 hr after the tumor implantation. The effect was evaluated in terms of the TPCV ratio on the 7th day after the tumor implantation. The results obtained are shown in Table II.

(ii) Activity Assay of Maltose- and Sucrose-[mono]-stearates upon Ehrlich Carcinoma by the Survival Method: The same administration schedule and method as described in ref. 1) were employed. Seven-day-old Ehrlich ascites carcinoma (about 1.5×10^6 cells/mouse) was implanted *i.p.* into female ICR strain mice. Each test sample was administered *i.p.* once daily for five consecutive days, starting 24 hr after the tumor transplantation, at various doses. The MST and ILS values obtained are shown in Table III.

(iii) Activity Assay of Maltose- and Sucrose-[mono]-stearates upon Solid Sarcoma 180: Sarcoma 180 ascites tumor (9.8×10^6 cells/mouse) was transplanted *s.c.* into the right groin of ICR female mice. Each test sample was administered *s.c.* in the back every other day, starting 24 hr after the tumor implantation, until the tenth injection. At the end of the fourth week, the mice were killed and the tumor weights were compared with those of the untreated controls. The inhibition ratios obtained are shown in Table IV.

(iv) Activity Assay of Maltose-[high]-esters and Sucrose-[mono]-stearate upon Solid Sarcoma 180: The general administration schedule and method were identical to those in (iii) with the following exceptions: inoculum, about 10^6 cells/mouse; animal, ddY mice (SPF, five-weeks-old); vehicle, physiological saline with 0.5% carboxymethylcellulose (CMC) (since the maltose-[high]-esters were sparingly soluble in normal saline, CMC was added to obtain a homogeneous suspension); administration route, *s.c.* and *i.m.* The results obtained are shown in Table V.

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References and Notes

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2) This paper deals with the combined results presented *a*) at the 48th General Meeting of the Japanese Biochemical Society, Fukuoka, October, 1975; *b*) at the 99th Annual Meeting of the Pharmaceutical Society of Japan, Sapporo, August, 1979.
3) Location: *a*) *13-1 Takara-machi, Kanazawa 920, Japan*; *b*) *5-1-1 Tsukiji, Chuo-ku, Tokyo 104, Japan.*
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