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Chemical and Biochemical Studies on Carbohydrate Esters. V.[1] Anti Ehrlich Ascites Tumor Effect and Chromatographic Behaviors of Fatty Acyl Monoesters of Sucrose and Trehalose[2]

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According to the procedure of Osipow, sucrose was partially acylated with palmitic, stearic, hydnocarpic, and ricinoleic acids. In the similar manner, the selective esterification of trehalose was performed, using caprylic, lauric, myristic, and stearic acids. The ester compositions of the resulting preparations were preliminarilly analysed by means of thin-layer chromatography and gas chromatography, and the major components contained in them were tentatively assigned to the 6-monoesters of these disaccharides. All the preparations obtained proved to exert considerable antitumor effect against Ehrlich ascites carcinoma in mice by intraperitoneal administration.

Keywords——antitumor effect; Ehrlich ascites carcinoma; sucrose monoesters of fatty acids; trehalose monoesters of fatty acids; Osipow's method; gas chromatography; thin-layer chromatography

In the preceding studies of this series, a number of fatty acids and the ester derivatives thereof were examined for their antitumor effect against Ehrlich ascites carcinoma implanted in mice.[5] Throughout the screening tests, the samples to be examined were administered by intraperitoneal injection for five consecutive days, and their activity was evaluated with the total packed cell volume (TPCV) ratio (%, treated/control) on the 7th day after the tumor implantation. From the results obtained, our particular attention has been attracted to the sucrose monoesters of fatty acids, since seven out of eight specimens so far tested tended to show marked antitumor effect, and, in addition, they were readily soluble in cold water.

In order to extend the above investigations, four additional examples of sucrose monoesters, as well as four kinds of fatty acyl monoesters derived from another non-reducing disaccharide, *i.e.*, α,α-trehalose (α-D-glucopyranosyl-α-D-glucopyranoside), have now been subjected to the similar antitumor bioassay at various doses. The samples employed were the sucrose monoesters of palmitic, stearic, ricinoleic (*d*-12-hydroxy-*cis*-9-octadecenoic), and hydnocarpic (11-(2-cyclopenten-1-yl) undecenoic) acids, and the trehalose monoesters of caprylic, lauric, myristic, and stearic acids.

The industrial products of sucrose monoesters can find diverse potential end uses, and hence extensive investigations of the esterification processes have been reported by many workers since 1956.[6] For preparation of our previous sucrose monoester specimens, we

1) Part IV: Y. Nishikawa and K. Yoshimoto, *Chem. Pharm. Bull.* (Tokyo), **25**, 624 (1977).
2) This work was presented partly; *a*) at the 95th Annual Meeting of the Pharmaceutical Society of Japan, Nishinomiya, April, 1975, and partly; *b*) at the 8th International Symposium on Carbohydrate Chemistry, Kyoto, August, 1976.
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5) *a*) Y. Nishikawa, M. Okabe, K. Yoshimoto, G. Kurono, and F. Fukuoka, *Chem. Pharm. Bull.* (Tokyo), **24**, 387 (1976); *b*) Y. Nishikawa, K. Yoshimoto, M. Okabe, and F. Fukuoka, *ibid.*, **24**, 756 (1976).
6) *a*) J.C. Colbert, "Sugar Esters, Preparation and Applications," (Chemical Technology Review No. 32), Noyes Data Corporation, Park Ridge and London, 1974; *b*) T. Ishizuka, *Yukagaku*, **21**, 408 (1972).

adopted the original Osipow's procedure, which was based on the reaction of sucrose with the methyl ester of a fatty acid, in *N*,*N*-dimethylformamide solution, using potassium carbonate as a catalyst.[7] Similarly, in the present study, partial acylation of both the disaccharides was carried out in this manner. The Osipow's method is, however, known to produce a complex mixture containing not only monoesters but also di-, tri-, and polyesters.[8] Using the products obtained from sucrose, compositional analyses have been attempted by various workers, but complete identification of the entire ester components produced in each case has not been accomplished as yet. However, most authors have proposed that, among the esters contained in the products, the sucrose-6-monoesters (the monoesters bearing their acyl radicals at the 6-position of the glucose portion) were prevailing, followed by the sucrose-6′-monoesters (the monoesters possessing their acyl functions at the 6-position of the fructose unit).[8a,b,g,n–p,9] Besides them, the sucrose-1′- and -4-monoesters and the sucrose-6,6′-diester have also been indicated to occur as minor constituents.[8g,n–p] On the other hand, the selective esterification of trehalose by application of the Osipow's method has never been studied previously.

In the present study, the respective reaction mixtures were purified in the following way, and, according to the three different purification stages, each test sample was designated as preparation A, B, or C. The reaction mixture was evaporated, and then washed with *n*-hexane for removal of the unreacted fatty acid methyl ester to give the crude preparation called A. Thin-layer chromatographic (TLC) analysis of the preparation A's derived from both the disaccharides was performed on silica gel plate with a mixed solvent of chloroform: methanol: acetic acid: water (79: 11: 8: 2) (solvent A), and the representative chromatograms obtained are depicted in Fig. 1. The TLC conditions were first recommended by Kinoshita for resolution of a monoester mixture of sucrose from its di-, tri- and polyesters.[8f,g] The four preparation A's derived from sucrose commonly showed the chromatographic features which were comparable with those reported in the literature.[8f,g] Thus, it proved evident that each of them contained principally a monoester mixture (the major spot having the smallest *Rf* value), accompanying with several higher esters (the minor spots having the larger *Rf* values). The preparation A's obtained from trehalose also gave mutually similar chromatograms. In general, the spot-patterns of the latters were simpler than those of the formers, probably due to the symmetric nature of the trehalose molecule. Considering from the chromatographic behaviors of the sucrose esters, the main spots possessing the lowest mobilities were assumed to be ascribable to the monoester mixtures of trehalose.

The preparation A obtained above was contaminated with the recovered disaccharide, either sucrose or trehalose, and the catalyst added. Extraction of the preparation A first

7) *a*) L. Osipow, F.D. Snell, W.C. York, and A. Finchler, *Ind. Eng. Chem.*, **48**, 1459 (1956); *b*) W.C. York, A. Finchler, L. Osipow, and F.D. Snell, *J. Am. Oil Chem. Soc.*, **33**, 424 (1956); *c*) L. Osipow, F.D. Snell, and A. Finchler, *ibid.*, **34**, 185 (1957); *d*) L. Osipow, S. Birsan, and F.D. Snell, *ibid.*, **34**, 34 (1957); *e*) L. Osipow, and F.D. Snell, *Intern. Sugar J.*, **59**, 68 (1957); *f*) *Idem*, *Chem. Prods.*, **20**, 101 (1957).

8) *a*) E. Reinefeld and S. Klaudianos, *Zucker*, **21**, 330 (1968); *b*) T. Otake and E. Tamate, *Kogyo Kagaku Zasshi*, **68**, 1896 (1965); *c*) *Idem*, *ibid.*, **67**, 809, 1586 (1964); *d*) W. Wachs and K. Gerhardt, *Tenside*, **2**, 6 (1965); *e*) F. Linow, H. Ruttloff, and K. Täufel, *Naturwissenschaften*, **50**, 689 (1963); *f*) 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; *g*) *Idem*, *Kogyo Kagaku Zasshi*, **66**, 450, 455 (1963); *h*) T. Ishikawa, *ibid.*, **66**, 715 (1963); *i*) H. Mima, N. Kitamori, and T. Kamizawa, *ibid.*, **65**, 833 (1962); *j*) H. Mima, and N. Kitamori, *J. Am. Oil Chem. Soc.*, **39**, 546 (1962); *k*) S. Komori, M. Okahara, and K. Okamoto, *ibid.*, **37**, 467 (1960); *l*) S. Komori, M. Okahara, and E. Shinsugi, *Kogyo Kagaku Zasshi*, **62**, 220 (1959); *m*) R.U. Lemieux and A.G. McInnes, *Can. J. Chem.*, **40**, 2394, 2376 (1962); *n*) M. Gee and H.G. Walker, *Anal. Chem.*, **34**, 650 (1962); *o*) *Idem*, *Chem. and Ind.*, **24**, 829 (1961); *p*) M. Gee, *J. Chromatog.*, **9**, 278 (1962); *q*) K. Mihara and K. Takaoka, *Kogyo Kagaku Zasshi*, **62**, 389 (1959).

9) The exceptional conclusion that 6-position was less reactive than 6′-position has been reported by Lemieux, *et al.*[8m]

with acetone and then with *n*-butanol, followed by evaporation of the combined extracts resulted in the further purified preparation named B, which was almost, if not entirely, free from those contaminants. By TLC examination, the ester-composition of each preparation B was revealed to be identical with that of the parent preparation A (Fig. 1).

Finally, the preparation B was chromatographed over silica gel column with solvent A, and the fraction corresponding to the major TLC-spot was collected to furnish the preparation C (Fig. 1). The acyl content of each preparation C was confirmed to be consistent with the theoretical value of the mono-substituted disaccharide ester. Otake has reported that the positional isomers of a sucrose monoester were separable satisfactorily by means of preparative TLC, when multiple developments were carried out on silica gel plate with a solvent system of chloroform: methanol (82: 18) (solvent B). Thus, the sucrose monoester mixture produced by the Osipow's procedure has been revealed to give five spots, among which the most and the second largest spots were proposed to be assignable to the 6- and 6′-isomers, respectively.[8b,c] Subsequently, the Otake's proposal was supported by Reinefeld.[8a] As shown in Fig. 2, our present preparation C's derived from sucrose commonly exhibited the TLC chromatograms whose features were similar to those mentioned by these authors (Fig. 2). Accordingly, they were considered to contain the sucrose-6-monoesters (I) as the major components.[10] Under these TLC conditions, the preparation C's obtained from trehalose

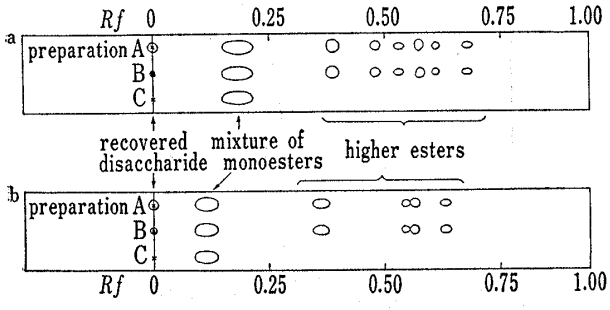


Fig. 1. TLC Chromatograms on Silica Gel with Solvent A

a : preparations derived from sucrose and stearic acid.
b : preparations derived from trehalose and stearic acid.

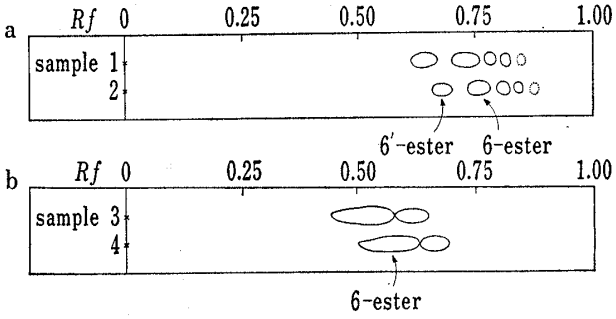
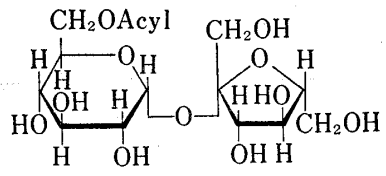


Fig. 2. TLC Chromatograms on Silica Gel with Solvent B (Triple-developments)

a : sample 1, preparation C derived from sucrose and myristic acid[5a].
2, preparation C derived from sucrose and stearic acid.
b : sample 3, preparation C derived from trehalose and myristic acid.
4, preparation C derived from trehalose and stearic acid.

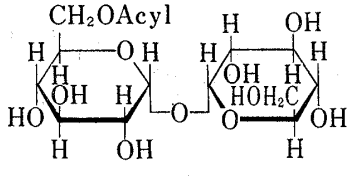


I

Acyl: palmitoyl, stearoyl, hydnocarpoyl

$\left(=\text{-}CO(CH_2)_{10}CH\begin{matrix}CH{=}CH\\ \diagup\quad\quad\diagdown\\ CH_2\text{-}CH_2\end{matrix}\right)$, or ricinoleoyl

$(=\text{-}CO(CH_2)_7CH{=}CHCH_2CH(OH)\text{-}(CH_2)_5CH_3)$



II

Acyl: capryloyl, lauroyl, myristoyl, or stearoyl

Chart 1

10) The conclusive evidences for the structure will be reported in a forthcoming paper of this series.

Table I. GLC Data of Preparation C's (as TMS Derivatives)[a]

Sample	Preparation C's derived from sucrose rt_R[b] (Area ratio, %)					
Palmitate	6.77(2.2)	7.69(1.9)	8.69(12.2)	9.85(69.4)	11.08(15.3)	
Stearate[c]	10.00(1.9)	11.23(2.0)	12.69(7.9)	14.69(71.0)	16.31(17.1)	
Hydnocarpate	7.63(trace)	8.94(1.6)	9.88(12.1)	11.50(69.8)	12.94(8.9)	16.69(7.7)
Ricinoleate	11.17(trace)	13.24(10.1)	15.59(4.1)	17.82(80.4)	20.00(5.4)	

Sample	Preparation C's derived from trehalose rt_R[d] (Area ratio, %)				
Caprylate	1.82(12.2)	2.12(8.2)	2.24(4.4)	2.79(75.3)	4.13(trace)
Laurate[c]	3.64(15.8)	4.18(4.4)	4.67(5.3)	5.58(74.4)	8.00(trace)
Myristate	5.09(11.3)	5.82(13.7)	6.55(2.7)	7.82(72.2)	11.39(trace)
Stearate	10.02(11.0)	11.42(9.2)	13.12(2.3)	14.84(77.6)	21.61(trace)

a) 1.5% OV-1 on Shimalite W (2 m×4 mm i.d.) at 295°; carrier gas, N_2 at 50—55 ml/min.
b) Internal standard: sucrose (as TMS derivative), 1.00 (1.3 min).
c) See Fig. 3.
d) Internal standard: trehalose (as TMS derivative), 1.00 (1.65 min).

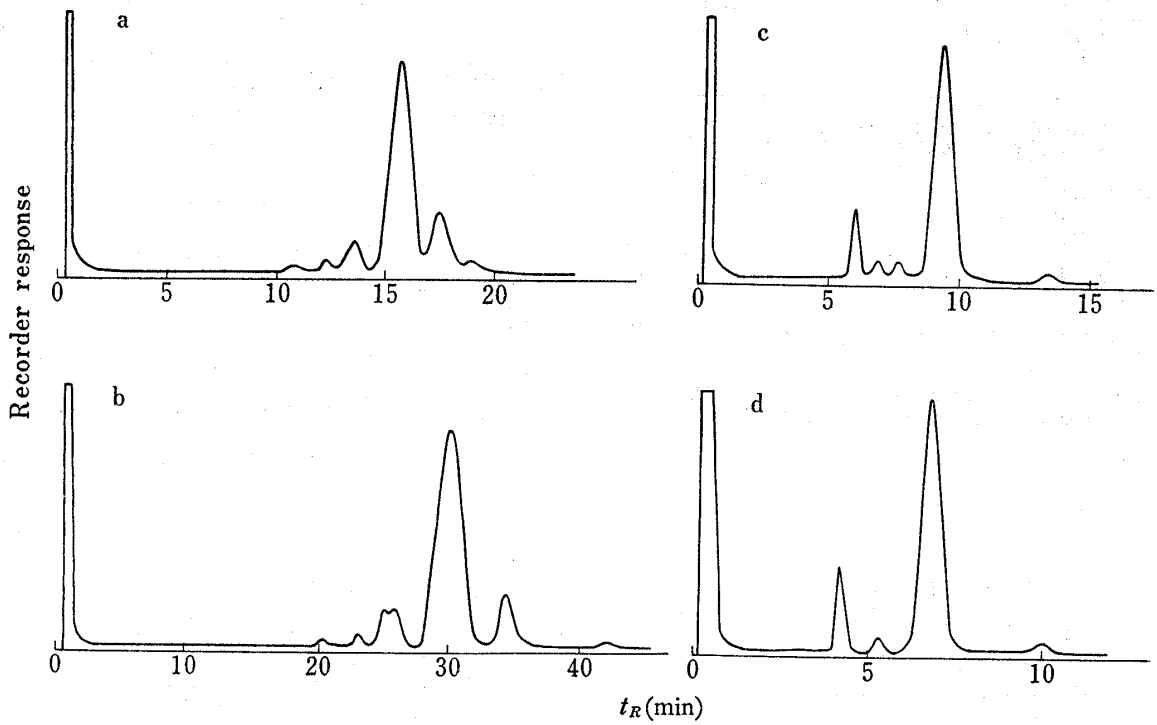


Fig. 3. GLC Chromatograms (as TMS Derivatives)

Fig. No.	Sample (preparation C)	Column packing	Column size	Column temp.	Flow rate of carrier gas (N_2)
a	Monostearates of sucrose	1.5% OV-1 on Shimalite W	2.0 m×4 mm i.d.	300°	60 ml/min
b	Monostearates of sucrose	1.5% OV-17 on Shimalite W	2.5 m×4 mm i.d.	295°	80 ml/min
c	Monolaurates of trehalose	1.5% OV-1 on Shimalite W	2.0 m×4 mm i.d.	295°	50 ml/min
d	Monolaurates of trehalose	1.5% OV-17 on Shimalite W	2.0 m×4 mm i.d.	295°	60 ml/min

were all found to show much simpler chromatograms each consisting of only two spots (Fig. 2). On the analogy of the results of the partial acylation of sucrose, the larger spots detected were assumed, tentatively, to be attributable to the trehalose-6-monoesters (II).[10] The Reinefeld's observations on the selective esterification of D-glucose may also support the above assumption.[11]

Hitherto, gas chromatography (GLC) has little been used for the direct compositional analysis of the products prepared by the Osipow's method.[5] We now examined the GLC behaviors of the preparation C's as their trimethylsilyl (TMS) derivatives, using OV-1 and OV-17 columns. In each case, no significant difference could be observed between the peak-patterns given by these two column packings. The chromatograms of the preparation C's derived from sucrose were mutually similar, each consisting of one large peak (area ratio, 70—80%) and several (four to five) minor peaks. Likewise, the preparation C's obtained from trehalose showed the chromatograms mutually resembling, each of which contained one large

Table II. Antitumor Effect against Ehrlich Ascites Carcinoma (TPCV Method)[a]

Sample	Prepn.[b]	Dose (mg/kg/day)	TPCV ratio(%) (Treated/Control)	Evaluation of activity[c]	Body wt. change (g)		Death/Total	
					Treated	Control	Treated	Control
i) Sucrose monoesters								
Palmitate	C	250×3[d]	1	##	−0.2	+2.0	3/6	0/5
	C	50×5	27	#	+0.5	+0.4	0/6	0/6
Stearate	C	250×5	2	##	−2.4	+2.0	0/6	0/5
	C	50×5	5	##	+1.3	+0.4	0/6	0/6
	C	50×5	30	#	+1.5	+3.3	0/6	0/6
	C	50×5	16	#	+2.0	+6.4	1/6	0/6
	C′	100×5	0	##	+2.6	+6.4	0/6	0/6
	C′	50×5	0	##	+4.1	+6.4	0/6	0/6
	C′	50×5	21	#	+1.2	+3.3	0/6	0/6
Hydnocarpate	B	400×5	20	#	−0.5	+2.3	1/6	0/6
Ricinoleate	B	400×5	17	#	−1.7	+2.3	1/6	0/6
	C	250×5	11	#	−0.1	−0.2	1/6	0/6
cf.)								
Caprylate	B[e]	400×5	36	#	−0.7	+0.5	4/6	1/6
Laurate	C[e]	250×5	25	#	−3.7	−0.6	4/6	0/6
Myristate	C[e]	250×5	0	##	−4.2	−0.6	5/6	0/6
	B[e]	100×5	36	#	−2.8	−1.4	1/6	0/6
	C	50×5	19	#	+1.1	+3.3	0/6	0/6
ii) Trehalose monoesters								
Caprylate	B	400×5	55	+	−2.2	+0.5	2/6	1/6
Laurate	A	400×5	7	##	+0.3	−0.03	0/6	0/6
	B	400×3[d]	1	##	−2.0	−0.03	3/6	0/6
	C	50×5	4	##	−1.9	+0.3	0/6	0/6
Myristate	A	400×5	53	+	−0.3	−0.03	0/6	0/6
	B	400×5	1	##	−0.4	−0.03	1/6	0/6
	C	250×3[d]	1	##	−3.5	+2.0	3/6	0/5
Stearate	C	250×1[d]	5	##	−3.2	+0.3	4/6	0/6
	C	100×5	0	##	−0.0	+0.3	0/6	0/6
	C	50×5	0	##	−1.0	+0.3	0/6	0/6

a) Route, *i.p.*; vehicle, N-saline; animal, ddY mice (female). The TPCV ratio, body wt. change, and death/total were determined on the 7th day after the tumor implantation.
b) Explanation on the preparations A, B, and C is given in the Text.
c) Evaluation ## # + −
TPCV ratio (% T/C) 0—10 11—40 41—65 66—100
d) Due to the strong toxicity of the sample, five injections were not attainable.
e) For comparison, the antitumor data were cited from ref. 5*a*).

11) E. Reinefeld and H.F. Korn, *Stärke*, **20**, 181 (1968).

peak (area ratio, 70—80%) and some (three to four) smaller peaks. The GLC data are listed in Table I, and the typical examples of the chromatograms are illustrated in Fig. 3. From these results, the preparation C's were indicated to be more heterogeneous than expected from their TLC analysis. Although the conclusive peak-identification has not been achieved as yet, the respective large peaks would be assignable to the 6-monoesters. In an attempt to establish a convenient GLC method useful for qualitative and quantitative determination of the monoester composition, further studies are now going on.

Most of the present antitumor experiments were performed by using the preparation C's which consisted solely of monoester isomers. But, in some cases, the crude preparations, A and B, which contained the higher esters in addition to the monoesters, were employed. Table II lists all the antitumor results obtained in the present study. For comparison and discussion, some data previously reported are also cited in Table II. The preparations tested now were found, without exception, to be effective in inhibiting the growth of Ehrlich ascites carcinoma. Especially remarkable activity was exhibited with the sucrose monoesters of palmitic, and stearic acids, as well as the trehalose monoesters of lauric, myristic, and stearic acids.

The finding that the present sucrose monoesters were all effective was not surprising, since many analogous examples derived from various common fatty acids, such as caprylic, lauric, myristic, elaidic, oleic, linoleic, and linolenic acids, had already been demonstrated to possess the similar activity.[5,12] However, it should be emphasized that the sucrose monoesters of two less common fatty acids, *i.e.*, ricinoleic and hydnocarpic acids, have now proved to be considerably effective. The observation may suggest the possibility that the sucrose monoesters having a wide variety of fatty acid moieties would be able to exert this type of antitumor effect. So far no example of the trehalose monoester produced by the Osipow's method has been revealed to be antitumor effective; though the fatty acid-trehalose ester derived from Arthrobacter had been evidenced to show a weak anti-Ehrlich ascites tumor effect, in spite of its strong activity upon sarcoma 180 solid tumor in mice.[13] The antitumor effect of the trehalose monoesters of four kinds of fatty acids has now been confirmed to be comparable to that of the sucrose monoesters of the corresponding fatty acids. Thus, it has been indicated that the alternation of the sugar moieties in the monoesters of these two non-reducing disaccharides would cause no significant influence on their antitumor activity.

Previously, we have assayed the antitumor effect of a number of fatty acids in the free form.[5] The results manifested that stearic acid was completely ineffective, and that lauric and myristic acids could exhibit the activity, only when they were administered at the doses higher than 300 mg/kg/day×5. In the present investigation, dose response assay was performed with some samples. From the results, it was established that the sucrose-monomyristate, the trehalose-monolaurate, and the monostearates of both the disaccharides maintained considerable activity even at the daily dose of 50 mg/kg. Accordingly, it became apparent that the antitumor action of this type of carbohydrate esters should not be explained by liberation of the antitumor effective fatty acids from the molecules: the esters themselves would act as active principle.

The commercially available sucrose esters of fatty acids are now widely used as food-additives, and their oral administration has been officially recognized to be non-toxic.[14] During the course of the present and the previous antitumor bioassays, occasional deaths were, however, encountered in the mice which received our ester preparations intraperitoneally at the doses higher than 250 mg/kg/day×5. Therefore, it is noteworthy to mention that intraperitoneal injection of the monoester specimens of sucrose, as well as of trehalose, at the

12) See the Arima's papers cited in ref. 5.
13) R. Kojima, *Nippon Saikingaku Zasshi*, **26**, 533 (1971).
14) F.A.O. Nutrition Meeting Report, No. 46A (1969).

daily dose of 50 mg/kg appeared to evoke no serious toxicity in the mice of treated group.[15]

Earlier workers have claimed that the esterification reaction by the Osipow's method could not guarantee the exactly reproducible composition of the product even under the fully-controlled conditions.[8m] Therefore, it seemed to be necessary to examine the fluctuations of the antitumor effect among the different batches prepared independently from the same starting materials. For this purpose, the specimen corresponding to the preparation C derived from sucrose and stearic acid was repeatedly prepared. In Table II, the resulting product is expressed as preparation C'. The activity of the latter preparation was confirmed to be as striking as that of the preparation C. Thus, it has been suggested that the possible slight variations of the ester composition would not affect significantly the antitumor effect.

As described above, the preparations employed in the present study were assumed to consist mainly of the 6-monoesters of sucrose or trehalose. Accordingly, their antitumor effect may be attributable largely, if not exclusively, to the presence of these esters. At present, however, the influence of the acyl location and the degree of substitution on the antitumor activity is still unknown. In order to solve these problems, further investigations are now under progress.

Experimental

Production of Fatty Acyl Monoester Preparations from Sucrose and Trehalose——The partial acylation of sucrose with the methyl esters of palmitic, stearic, ricinoleic, and hydnocarpic acids, as well as of trehalose with the methyl esters of caprylic, lauric, myristic, and stearic acids was performed according to the Osipow's method.[7] The starting materials employed were purchased from commercial sources with one exception of the methyl hydnocarpate, which was prepared by methylation of the parent fatty acid freshly isolated from the Hydnocarpus oil. Before use, each reagent was analysed by means of GLC to guarantee the satisfactory purity. For production of the monoester preparations, the following general procedures were adopted. The disaccharide (45 mmol), either sucrose or trehalose, was dissolved in N,N-dimethylformamide (60 ml), and the methyl ester of an appropriate fatty acid (15 mmol) was added with K_2CO_3 (1.5 mmol) as an alkaline catalyst. The reaction mixture was maintained at 90—95° under 80—100 mmHg pressure with stirring in the stream of N_2 for 9 hr. Then the solvent was distilled off at 80—85° under diminished pressure (30—40 mmHg). The residue was washed thoroughly with *n*-hexane for removal of the unreacted methyl ester of fatty acid, and then dried over silica gel in a desiccator to give the preparation A as a slightly brownish amorphous powder. The preparation A was first extracted with hot acetone (150 ml × 3). Successively, the residue was heated with *n*-BuOH (300 ml) on a boiling water-bath, and, while hot, the undissolved materials (the catalyst and the recovered disaccharide) were filtered off. Both the extracts were combined together, and evaporated to dryness *in vacuo* to yield the preparation B as an almost colorless, slightly hygroscopic powder. Yield from the methyl ester of fatty acid, 70—75%. The preparation B was chromatographed over silica gel column with solvent A. Monitoring by TLC, the fractions corresponding to the spot of the monoester mixture were collected to furnish the preparation C as a colorless, slightly hygroscopic powder. Yields from the methyl ester of fatty acid, 45—55%. All the preparation C's thus obtained were readily soluble in water (foaming). They were also readily soluble in MeOH, EtOH, AcOH, pyridine, and N,N-dimethylformamide, and considerably in *n*-BuOH, acetone, and $CHCl_3$, but insoluble in ether, benzene, *n*-hexane, and CCl_4.

TLC Analysis——Throughout the present work, TLC analyses were carried out on Silica Gel G (Merck) layer (length, 20 cm; thickness, 0.25 mm), and the spots were detected with 50% H_2SO_4 reagent. Two solvent systems, A and B, whose compositions are mentioned in the Text, were employed for developments. The former system was applied to the analyses of all the preparations obtained in the present study (single development), while the latter only to those of the preparation C's (triple developments). The resulting chromatographic features are described in the Text, and the representative chromatograms are depicted in Fig. 1 and 2. The *Rf* values of the spots observed were as follows. i) Preparation A's derived from sucrose: palmitate, 0.13—0.20 (major spot), 0.37, 0.45, 0.50, 0.55, 0.59, and 0.65 (minor spots); stearate, 0.15—0.21 (major spot), 0.39, 0.48, 0.53, 0.58, 0.61, and 0.68 (minor spots); hydnocarpate, 0.04—0.11 (major spot), 0.20, 0.27, 0.33, 0.39, and 0.48 (minor spots); ricinoleate, 0.04—0.13 (major spot), 0.19, 0.27, 0.33, 0.39, and 0.46 (minor spots). Besides them, the spot due to the recovered sucrose was commonly detected at *Rf* 0.00 with considerable intensity. ii) Preparation A's derived from trehalose: caprylate, 0.03—0.09 (major spot),

15) As expected, the mice treated with the sucrose monoester of ricinoleic acid had diarrhea.

0.21, 0.34, 0.36, and 0.42 (minor spots); laurate, 0.05—0.10 (major spot), 0.29, 0.44, 0.46, and 0.53 (minor spots); myristate, 0.05—0.11 (major spot), 0.32, 0.48, 0.50, and 0.55 (minor spots); stearate, 0.07—0.14 (major spot), 0.37, 0.56, 0.58, and 0.62 (minor spots). Besides them, the spot due to the recovered trehalose was commonly detected at *Rf* 0.00 with considerable intensity. iii) Preparation B's derived from both the disaccharides: the spot-patterns were almost identical with those of their parent preparation A's except that the spot-intensities of the recovered disaccharides were significantly diminished. iv) Preparation C's derived from both the disaccharides (solvent A): each of them gave a single spot corresponding to the major spot observed in the chromatogram of its parent preparation A. v) Preparation C's derived from sucrose (solvent B, triple-developments): palmitate, 0.66, 0.74 (I), 0.79, 0.83, and 0.86; stearate, 0.68, 0.76 (I), 0.81, 0.84, and 0.88; hydnocarpate, 0.54, 0.62 (I), 0.67, 0.71, and 0.73; ricinoleate, 0.53, 0.60 (I), 0.65, 0.69, and 0.71. vi) Preparation C's derived from trehalose (solvent B, triple-developments): caprylate, 0.33—0.42 (II), and 0.45; laurate, 0.42—0.52 (II), and 0.56; myristate, 0.44—0.57 (II), and 0.62; stearate, 0.49—0.63 (II), and 0.66.

GLC Analysis——The preparation C's derived from both the disaccharides were converted into the TMS derivatives by the method of Sweeley,[16] and then analysed with a Shimadzu Gas Chromatograph Model GC-4BPF attached with a hydrogen flame ionization detector, using glass columns. The relative retention times (rt_R) and the representative chromatograms are shown in Table I and in Fig. 3, respectively, together with the operation conditions employed.

Determination of Acyl Contents——The acyl contents of all the preparation C's obtained were estimated according to the following procedures. A mixture of a test sample and an appropriate fatty acid used as an internal standard was heated with 3% MeOH–HCl at 100° for 2 hr in a sealed tube. Using the ether extract of the reaction mixture, the amount of the fatty acid methyl ester liberated from the sample was determined by means of GLC in the usual manner. The values found were as follows. i) Preparation C's derived from sucrose: palmitate, 39.7% (41.2%); stearate, 41.3% (43.9%); hydnocarpate, 38.2% (40.8%); ricinoleate, 42.8% (45.2%). ii) Preparation C's derived from trehalose: caprylate, 26.3% (27.2%); laurate, 34.2% (35.0%); myristate, 36.9% (38.2%); stearate, 42.5% (43.9%). (The figures given in parentheses are the theoretical values calculated as the monoesters.).

Antitumor Bioassay (TPCV (Total Packed Cell Volume) Method)[17]——The Ehrlich ascites tumor cells (7×10^6 cells/mouse) were implanted intraperitoneally in ddY mice (female), weighing 23 ± 2 g. The agent to be tested was dissolved in N-saline. Treatment was initiated 24 hr after tumor implantation, the agent being given by intraperitoneal injection once daily for five consecutive days. On the 7th day after the tumor implantation, the antitumor activity was evaluated with TPCV (TPCV (ml) = volumes of ascites (ml) × ascitocrit) ratio (%, treated/control). All the antitumor results obtained in the present study are listed in Table II.

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