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# Chemical and Biochemical Studies on Carbohydrate Esters. VIII.[1] Antitumor Activity of Sucrose Fatty Acid Esters[2]

Tetsuro Ikekawa, Mari Umeji (née Ohtsuka), Syun'suke Yanoma,[3a] Kimihiro Yoshimoto, and Yoshihiro Nishikawa[3b]

*National Cancer Center Research Institute,[3a] and Faculty of Pharmaceutical Sciences, Kanazawa University[3b]*

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The monoester compositions of nine new sucrose fatty acid ester preparations produced industrially, which contain mono-, di-, and polyesters of stearic and palmitic acids in different ratios, were quantitatively analyzed by gas-liquid chromatography.

Monoester-rich preparations had a marked cell growth inhibiting effect upon L-5178Y, as determined by the tissue culture method. It was observed that the $ID_{50}$ values tend to decrease with increase in HLB (hydrophile-lipophile balance) values. These preparations were effective against Ehrlich ascites carcinoma as determined by the total packed cell volume method. Preparations consisting mainly of monoesters increased the life spans of mice bearing Ehrlich ascites tumor, but were not effective against L-1210.

**Keywords**——sucrose fatty acid esters; antitumor effect; gas liquid chromatography; tissue culture method; total packed cell volume method; survival method; cell growth inhibitory effect

In the previous studies of this series, we have examined the antitumor activities of various fatty acid ester preparations of sucrose and trehalose by using *in vivo* and *in vitro* bioassay methods.[4] It has been observed that certain monoester preparations of these disaccharides exhibit remarkable antitumor effects against Ehrlich ascites carcinoma, when assayed by the total packed cell volume (TPCV) method.[4b-d,5] These esters were prepared according to the original procedure of Osipow,[6] followed by column chromatographic fractionation of the crude reaction products. A sucrose-monostearate preparation[4b,d] and DK-esters, that is, sucrose esters produced industrially from hydrogenated beef tallow fatty acids by the improved Nebraska-Snell process,[7] were also found to be highly effective in inhibiting the tumor growth of a mouse leukemia cell line, L-5178Y, by the tissue culture method.[4a] Furthermore, preliminary results indicated that when tested by the TPCV method the sucrose-monostearate preparation showed an antitumor effect upon ascites sarcoma 180.[4a]

Recently, a second line of industrial sucrose esters, the Ryoto sugar ester (RS-ester) has appeared in Japan.[8] For production of this new commercial sucrose ester, an advanced

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2) This work was presented at the 98th Annual Meeting of the Pharmaceutical Society of Japan, Okayama, April, 1978.
3) Location: *a) 5-1 Tsukiji, Chuo-ku, Tokyo 104, Japan*; *b) 13-1 Takara-machi, Kanazawa 920, Japan.*
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8) *a)* T. Kosaka and T. Yamada, "Sucrochemistry," (ACS Symposium Series No. 41), ed. by J.L. Hickson, *Am. Chem. Soc.*, Washington D.C., 1977, p. 84; *b)* Monographs distributed by Ryoto Co., Ltd.

Table I. Analytical Properties of RS-ester Preparations

| Type of RS-ester | HLB value[a] | Ester composition (%)[b] | | | Monoester composition (mg in 100 mg of RS-ester)[c] | | | |
|---|---|---|---|---|---|---|---|---|
| | | mono- | di- | poly- | Stearate | Palmitate | Myristate | Total |
| S-370 | 3 | 19 | 34 | 47 | 12.1 | 5.6 | 0.5 | 18.2 |
| S-570 | 5 | 31 | 37 | 32 | 15.7 | 7.2 | 0.7 | 23.6 |
| S-770 | 7 | 40 | 36 | 24 | 27.0 | 14.1 | 1.0 | 42.1 |
| S-970 | 9 | 51 | 34 | 15 | 28.9 | 15.2 | 1.4 | 45.5 |
| S-1170 | 11 | 58 | 31 | 11 | 36.0 | 20.3 | 1.1 | 57.4 |
| S-1570 | 14 | 71 | 24 | 5 | 51.4 | 22.9 | 1.3 | 75.6 |
| M-90[d] | 18 | 93 | 7 | 0 | 54.8 | 35.1 | 2.6 | 92.5 |
| P-1570 | 15 | 69 | 25 | 6 | 10.9 | 60.5 | 0.7 | 72.1 |
| P-1670 | 16 | 80 | 18 | 2 | 13.1 | 62.3 | 0.8 | 76.2 |

*a*) The data were identical with those reported by the manufacturer.[8]
*b*) Manufacturer's data obtained by liquid chromatography.[8]
*c*) Data obtained in our laboratory by GLC.
*d*) A sample specially provided by the research institute of Ryoto Co., Ltd.

Osipow process is employed.[9] As shown in Table I, there are several types of RS-ester preparations differing in ester composition.

In the present study, the monoester compositions of these RS-ester preparations were analyzed by gas-liquid chromatography (GLC), and their antitumor activities were examined using the same bioassay methods as were used previously for the DK-esters. We studied in detail the cell-growth inhibitory effects of these sucrose ester preparations by the tissue culture method using a mouse leukemia cell line, L-5178Y, and determined the $ID_{50}$ value for each specimen. In the present study we have attempted to correlate the cell growth inhibitory effect and the chemical composition of the samples tested and to find some relationship between their $ID_{50}$ and HLB (hydrophile-lipophile balance) values. We also report here the results of survival tests performed with Ehrlich ascites carcinoma and L-1210 using the sucrose-monostearate preparation and some of the DK- and RS-esters as test samples.

## Experimental

**Material**——i) RS-Ester: The nine types of RS-ester used and their analytical properties are presented in Table I. These were obtained from the manufacturer (Ryoto Co., Ltd., Tokyo, Japan) and were used without further treatment. Their monoester compositions were analyzed by GLC under the conditions reported for the DK-esters.[4a] Identification of the peaks and their quantitative estimation were carried out using corresponding authentic sucrose-monoester specimens prepared previously.[4b,d]

ii) DK-Ester F-160: This was supplied by the manufacturer (Dai-ichi Kogyo Seiyaku Co. Ltd., Kyoto, Japan), and was used without further purification. Its analytical data were reported earlier.[4a]

iii) Sucrose-Monostearate Preparation: This was prepared as described previously.[4b,d]

iv) 5-FU: This agent was purchased from Sigma Chemical Co., U.S.A.

**Methods**——i) Assay with L-5178Y by the Tissue Culture Method: The tests were carried out as reported in our previous papers.[4a] The leukemia cells were cultured in a stoppered tube, using RPMI-1640 medium supplemented with 10% fetal calf serum at 37°. The growth inhibitory effect was determined as the ratio of cell numbers in treated and control groups (% T/C) after incubation of *ca.* $2.0 \times 10^5$ cells/ml for 48 hr with various concentrations of the test agent ranging from 500 to 15.625 μg/ml. The cell numbers were generally counted visually with a microscope. A Toa microcell counter (model CC-108) was used, especially in the initial screening test, and the results were found to be almost identical with those obtained by the microscopic measurements. To express the inhibitory effects of individual agents, their $ID_{50}$ values were determined by a probit diagraming analysis, namely by transforming the cell growth inhibition percentages into probit data.

ii) Assay with Ehrlich Ascites Carcinoma by the TPCV Method:[5a,b] The general bioassay procedures employed were similar to those adopted in our previous studies. Seven-day-old Ehrlich ascites carcinoma

9) Details of the new process have not yet been disclosed.

(about $10^7$ cells/mouse) was inoculated intraperitoneally (*i.p.*) into female ICR strain mice weighing $22 \pm 2$ g. The test sample was dissolved or suspended in 0.9% saline with or without 0.2% Tween 80, and injected *i.p.* once daily for five consecutive days, starting 24 hr after tumor implantation, at a standard dosage of 250 mg/kg/day $\times$ 5 days. The effect was evaluated in terms of the TPCV ratio (% T/C) on the 7th day after tumor implantation.

iii) Assay with Ehrlich Ascites Carcinoma by the Survival Method: Seven-day-old Ehrlich ascites carcinoma (approximately $10^7$ cells/mouse) was implanted *i.p.* into female ICR mice. Each test sample was dissolved in 0.9% saline containing 0.2% Tween 80 and administered *i.p.* once daily for five consecutive days, starting 24 hr after tumor transplantation, at a daily dose of 250 mg/kg or 100 mg/kg, and the life spans of the treated group were compared with those of the untreated mice.

iv) Assay with L-1210 by the Survival Method: The general administration schedule and method were identical with those in iii), except that female $BDF_1$ mice *i.p.* implanted with L-1210 (approximately $3 \times 10^5$ cells/mouse) were treated with the test agent at a dose of 250 mg/kg/day $\times$ 5 days.

v) Combination Therapy Against L-1210 and P-388: The tumor cells, L-1210 ($3.0 \times 10^5$ cells/mouse) or P-388 ($1.1 \times 10^6$ cells/mouse), were inoculated *i.p.* into female $BDF_1$ mice. Three sorts of test sample, that is, 5-FU (30 mg/kg) in distilled water, 5-FU (30 mg/kg) in 5% aqueous solution of RS-ester S-1570, and 5% aqueous solution of RS-ester S-1570, were administered orally into three groups of mice for five consecutive days, starting 24 hr after tumor transplantation. The life spans of the treated groups were compared with those of the control mice.

## Results and Discussion

The analytical properties of the nine types of RS-ester used as test samples in the present study are summarized in Table I. They contain mono-, di-, and polyester components in different proportions, and their HLB values increase with increasing monoester content. The monoester compositions of the RS-esters were determined by GLC analysis. The gas

Table II. Cell Growth Inhibitory Effect of RS-ester Preparations upon L-5178Y (Tissue Culture Method)

| Type of RS-ester | $ID_{50}$ (μg/ml) | Type of RS-ester | $ID_{50}$ (μg/ml) |
|---|---|---|---|
| S-370 | 360 | S-1570 | 86 |
| S-570 | 285 | M-90 | 50 |
| S-770 | 132 | P-1570 | 53 |
| S-970 | 95 | P-1670 | 51 |
| S-1170 | 112 | | |

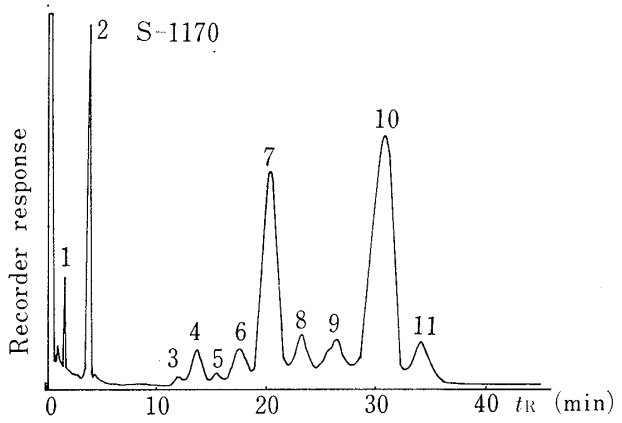


Fig. 1. Gas Chromatogram of RS-ester (as TMS-Derivative)[a] 1, sucrose; 2, cholesterol (internal standard); 3, 4[b], 5, monoesters of myristic acid; 6, 7[b], 8, monoesters of palmitic acid; 9, 10[b], 11, monoesters of stearic acid.

*a*) 1.5% OV-1 on Shimalite W (2 m $\times$ 3 mm I.D.), at 295°.
*b*) The peak tentatively identified as that of the 6-ester.

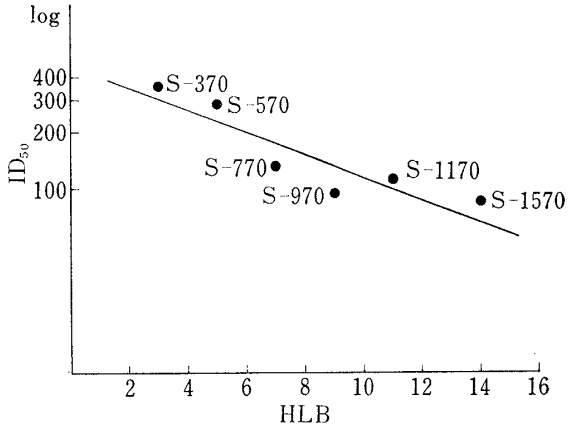


Fig. 2. Relationship between log $ID_{50}$ and HLB Values

chromatograms were all qualitatively similar, consisting of peaks identified as the monoesters of stearic, palmitic, and myristic acids. The presence of small quantities (less than 10%) of unconverted sucrose was also commonly detected (Fig. 1). Quantitative analysis showed that the RS-ester specimens belonging to the S- and M-series are mainly composed of stearoyl esters, while those of the P-series contain chiefly palmitoyl esters, accompanied by considerable amounts of palmitates and stearates, respectively. Traces of myristates were found in all the samples (Table I). Satisfactory agreement was observed between the total monoester contents obtained by GLC analysis and the corresponding liquid chromatographic data supplied by the mannufacturer[7]. In addition, comparison of the analytical data for RS-ester with those for DK-ester reported earlier[4a] indicates that there is a close similarity in the ester compositions of the two series of commercial products, the RS-ester preparations S-370, -570, -770, -970, -1170, and -1570 resembling the DK-ester preparations of type F-20, -50, -70, -90, -110, and -160, respectively.

The anti-leukemia effect of the RS-esters with L-5178Y was evaluated by the tissue culture method, and the results are presented in Table II. As reported earlier, preliminary results on the inhibitory effects of various types of DK-ester examined by the same method indicated that the DK-esters with higher monoester contents showed cell growth inhibition at a concentration of 500 $\gamma$/ml, whereas those with lower monoester contents were ineffective.[4a] Based on this preliminary finding it has been suggested that certain monosubstituted components may be responsible for the anti-leukemia activity. To confirm these findings, we have now evaluated in detail the anti-leukemia effect of the RS-ester by determining their $ID_{50}$ values in cell growth inhibition. As shown in Table II, the $ID_{50}$ values tend to decrease with increase of the monoester contents, though there is a slight discrepancy in the case of type S-1170. As shown in Table I, the HLB values increase with increase of the monoester content. Therefore, these findings suggest some correlation between the *in vitro* anti-leukemia effect and the HLB values in the sucrose fatty acid ester preparations. It is also noteworthy that there is an approximately linear relationship between the log $ID_{50}$ and HLB values in

Table III. Antitumor Effect of RS-ester Preparations upon Ehrlich Ascites Carcinoma (TPCV Method)

| Type of RS-ester | Dose (mg/kg/day × days) | TPCV ratio (%,T/C) | Evaluation of activity[a] | Body wt. change (g) | Deaths/total |
|---|---|---|---|---|---|
| Experiment 1[b] | | | | | |
| S-370 | 250×5 | 0 | ## | −4.7 | 2/6 |
| S-570 | 250×5 | 1 | ## | −5.6 | 0/6 |
| S-770 | 250×5 | 1 | ## | −5.9 | 0/6 |
| S-970 | 250×5 | 1 | ## | −5.6 | 0/6 |
| S-1170 | 250×5 | 1 | ## | −5.7 | 0/6 |
| S-1570 | 250×5 | 1 | ## | −4.9 | 0/6 |
| Control | | | | +2.2 | 0/5 |
| Experiment 2[c] | | | | | |
| P-1570 | 250×5 | 15 | # | −1.0 | 0/6 |
| P-1670 | 250×5 | 0 | ## | −1.3 | 0/6 |
| Control | | | | +0.3 | 0/6 |
| *cf.* | | | | | |
| Sucrose-monopalmitate[d] | 50×5 | 27 | # | +0.5 | 0/6 |
| Sucrose-monostearate[d] | 250×5 | 2 | ## | −2.4 | 0/6 |
| | 50×5 | 5 | ## | +1.3 | 0/6 |
| DK-ester F-90[e] | 250×5 | 1 | ## | −2.9 | 0/6 |

*a*) Oriterion (%, T/C): ## (0—10), # (11—40), + (41—65), − (66—100).
*b*) Animal, femal ICR mouse; vehicle, 0.9% saline,; route, *i.p.*
*c*) Animal, female ICR mouse; vehicle, 0.9% saline with 0.2% Tween 80; route, *i.p.*
*d*) Data cited from ref. 4*b*). aminal, female ddY mouse; vehicle, 0.9% saline; route, *i.p.*
*e*) Data cited from ref. 4*a*). animal, female ddY mouse; vehicle, 0.9% saline; route, *i.p.*

the RS-ester specimens belonging to the S-series, as shown in Fig. 2.

As shown in Table III, all types of RS-ester tested exhibited marked antitumor effects against Ehrlich ascites carcinoma in mice as determined by the TPCV method. The dosage adopted was identical with that used for the DK-esters. The results were not unexpected in view of our previous findings. Instead of the ddY strain mice employed in our previous experiments,[4] ICR strain mice were used in the present study, but this change did not significantly affect the experimental results in this antitumor bioassay. Based on the *in vitro* and *in vivo* experimental results described above, four samples, namely the sucrose monoester preparation[4b,d] DK-ester F-160,[4a] and RS-ester S-1570 and P-1670[8] were selected, and survival tests were performed with Ehrlich ascites carcinoma and L-1210. As shown in Table IV, they caused a significant increase of the life span in mice bearing Ehrlich ascites tumor on *i.p.* injection. The reason why they were more effective at the lower dose is not clear, but it is conceivable that *i.p.* administration at the higher dosage results in higher toxicity. On the other hand, these specimens were all found to be ineffective against L-1210 at a daily dose of 250 mg/kg.

Table IV. Antitumor Effects of Sucrose Ester Preparations upon Ehrlich Ascites Carcinoma (Survival Method)

| Test-sample | 250 mg/kg/day × 5 | | 100 mg/kg/day × 5 | |
|---|---|---|---|---|
| | MST(days) | ILS(%) | MST(days) | ILS(%) |
| Sucrose-monostearate preparation | 24.5 | 116 | 27.7 | 177 |
| DK-ester F-160 | 25.5 | 125 | 23.3 | 133 |
| RS-ester S-1570 | 21.0 | 85 | 19.6 | 96 |
| RS-ester P-1670 | 20.8 | 84 | >37.0 | >270 |
| Control | 11.3 | | 10.0 | |

Animal, female ICR mouse; vehicle, 0.9% saline with 0.2% Tween 80; route, *i.p.*
MST: mean survival time, ILS: increase in life span.

We also attempted combination therapy with 5-FU and RS-ester of type S-1570 (oral administration), using L-1210 and P-388 tumor cells. However, no synergistic effect was obtained in these experiments.

In spite of the efforts of earlier workers, a complete analysis of the components contained in sugar ester prepared by Osipow's method has not yet been achieved due to their complexity. Moreover, the composition of the products is unlikely to be exactly reproducible between production runs. The three lines of sucrose ester specimens so far used for antitumor assays, namely our monoester preparations, the DK-esters and the RS-esters, were prepared under somewhat different conditions, but gross similarity of their monoester compositions was indicated by GLC analyses. The results of our previous and present studies show that both types of commercial preparations with higher monoester contents can exhibit pronounced antitumor effects in various assays, in spite of small compositional variations. It appears that higher monoester contents of these sugar fatty acid esters result in higher antitumor activities, and the possible compositional variations arising from slight differences in the synthetic conditions did not significantly affect the results in various antitumor bioassays.

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