are devoid of ABA-like ability. Thus, it was concluded that the presence of the lauroyl function attached to the 1-OH group, either axial or equatorial, of the D-glucose unit is critical for this biological effect. In view of this conclusion, the previously observed inactivity of the sucrose- and the trehalose-monoester of lauric acid[4] was considered to be attributable to inappropriate locations of the acyl function introduced: sucrose and trehalose are non-reducing disaccharides and hence they lack free anomeric-OH groups to be acylated. This view was, however, ruled out by the observation that maltose-laurate preparations, which, as reported preliminarily, contained 1-substituted components,[1] also proved to be completely inactive. The inactivity of the sucrose-, trehalose, and maltose-esters of lauric acid may be accounted for by the dimeric nature of their carbohydrate units. In other words, monomeric nature of the carbohydrate residue has now been demonstrated to be another important factor in the ABA-like activity of 1-O-lauroylglucose.

So far, little is known about fatty acid esters of carbohydrates with plant hormonal effects. However, two interesting papers have been published during the course of this work. Grove *et al.*[7] reported that an active principle of "brassin" was a steroidal compound termed brassinolide. As cited in our preceding paper,[4] the active components contained in "brassin," which was first isolated from *Brassica napus* (rape) pollen in 1970 and shown to possess a novel plant growth promoting effect, were tentatively identified as the 1-glucosyl esters of fatty acids by Mandava *et al.*, though this conclusion was questioned by Milborrow *et al.* In 1979, Tanaka *et al.*[8] showed that synthetic 1-O-palmitoyl-, 1-O-oleoyl-, and 3-O-linolenoyl-D-glucopyranoses failed to exert "brassin"-type activity, but found that the last compound had a promoting effect on the germination of pollen and the growth of pollen tubes.

References and Notes

1) Part IX: Y. Nishikawa, K. Yoshimoto, M. Nishijima, F. Fukuoka, and T. Ikekawa, *Chem. Pharm. Bull.*, **29**, 505 (1981).
2) Presented at the General Meeting of the Agricultural Chemical Society of Japan, Tokyo, April, 1979.
3) Location: *a*) *13-1 Takara-machi, Kanazawa 920, Japan*; *b*) *1-1 Marunouchi, Kanazawa 920, Japan.*
4) Y. Nishikawa, K. Yoshimoto, and M. Ohkawa, *Chem. Pharm. Bull.*, **27**, 2011 (1979).
5) J.P. Nitsch and C. Nitsch *Plant Physiol.*, **31**, 94 (1956).
6) *a*) Y. Nishikawa and K. Yoshimoto, *Chem. Pharm. Bull.*, **25**, 624 (1977); *b*) K Yoshimoto, K. Tahara, S. Suzuki, K. Sasaki, Y. Nishikawa, and Y. Tsuda, *ibid.*, **27**, 2661 (1979).
7) M.D. Grove, G.F Spencer., W.K. Rohwedder, N. Mandava, J.F. Worley, J.D. Warthen, Jr., G.L. Steffens, J.L. Flippen-Anderson, and J. Carter Cook, Jr., *Nature*, **281**, 216 (1979).
8) A.K. Tanaka, A. Kobayashi, and K. Yamashita, *Agric. Biol. Chem.*, **43**, 2537 (1979).

[Chem. Pharm. Bull. 29(3) 880—883 (1981)]

Chemical and Biochemical Studies on Carbohydrate Esters. XI.[1] Antitumor Effects of Fatty Acid Monoesters of D-Glucose[2]

YOSHIHIRO NISHIKAWA,*[,3a] KIMIHIRO YOSHIMOTO,[3a] KUMIKO ASHIZAWA,[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

(Received August 11, 1980)

1-O-, 3-O-, and 6-O-acyl-D-glucopyranoses carrying capryloyl, lauroyl, myristoyl, palmitoyl, and stearoyl functions were tested for their *in vivo* and *in vitro* antitumor

effects against Ehrlich ascites carcinoma in mice and a leukemia cell line L-5178Y by the total packed cell volume method and the tissue culture method, respectively. In the *in vivo* bioassay, the 1- and 3-myristate and the 6-stearate were moderately effective, while all other compounds exhibited little or no effect. These samples inhibited the growth of the cultured leukemia cells to various extents: relatively lower ID_{50} values were obtained with the 1-laurate, 1-myristate, 1-palmitate, 3-myristate, and 6-laurate. On the basis of these findings, the monoesters of monosaccharide appear to be less promising as antitumor agents than the analogous derivatives of disaccharide.

Keywords——1-O-acyl-D-glucopyranose; 3-O-acyl-D-glucopyranose; 6-O-acyl-D-glucopyranose; total packed cell volume method; tissue culture method; antitumor effect; Ehrlich ascites carcinoma; L-5178Y

In the previous studies of this series, various disaccharide esters of long chain fatty acids were examined for *in vivo* and *in vitro* antitumor effects.[4] Some of the samples tested were found to possess significant antitumor activities against certain tumors such as Ehrlich ascites carcinoma, sarcoma 180, and L-5178Y. In the present work, we undertook antitumor bioassays of mono-O-acyl-D-glucopyranoses in order to compare the antitumor activities of monoesters of mono- and disaccharides. Three series of positional isomers of D-glucosyl monoesters, that is, 1-, 3-, and 6-esters, of caprylic, lauric, myristic, palmitic, and stearic acids were chosen as test samples. They were synthesized according to the procedures reported in our preceding papers[5]: in the case of 1-esters, the samples used consisted almost exclusively of the α-anomers. The *in vivo* antitumor effects of these samples were evaluted by the total packed cell volume (TPCV) method against Ehrlich ascites carcinoma in mice. Their *in vitro* antitumor activities upon a mouse leukemia cell line, L-5178Y, were determined by the tissue culture method. Both bioassays were carried out under the same conditions as before.[4]

Materials and Methods

Test Samples——1-O-, 3-O-, and 6-O-Acyl-D-glucopyranoses (acyl: capryloyl, lauroyl, myristoyl, palmitoyl, and stearoyl) were prepared as described in our previous papers,[5] and their physical properties were confirmed to be identical with those of the corresponding authentic specimens.

Bioassay Method——(i) TPCV Method:[6] Seven-day-old Ehrlich ascites carcinoma (about 10^7 cells/mouse) was inoculated intraperitoneally (*i.p.*) into female ddY strain mice (six mice were used for each test sample). A suspension of test sample in normal saline was injected *i.p.* once daily for five consecutive days, starting 24 hr after the tumor implantation. The effect was evaluated in terms of the TPCV ratio (%, treated/control) on the 7th day after the tumor transplantation. The results were expressed in terms of the same criteria as employed before.

(ii) Tissue Culture Method:[7] The leukemia cells were cultured in a stoppered tube, in RPMI-1640 medium supplemented with 10% fetal calf serum at 37°. The growth inhibitory effect was determined as the ratio of cell numbers, which were counted visually with a microscope, in treated and control groups (%, treated/control) after incubation of *ca.* 2.0×10^5 cells/ml for 48 hr with various concentrations of each test agent ranging from 500 to 31.25 μg/ml. To express the results, the ID_{50} (50% inhibiting dose) value was calculated by a probit diagraming analysis.

Results and Discussion

The antitumor activities of fourteen D-glucosyl monoesters, as evaluated in the TPCV bioassay by *i.p.* injection at the dose of 250 mg/kg/day×5, are shown in Table I. For comparison, our previous data[4] obtained with the disaccharide-monoesters under similar bioassay conditions are also presented in Table I.

Most of the samples examined proved to have little or no effect upon this asscites tumor, but three specimens, that is, 1-O- and 3-O-myristoyl- and 6-O-stearoyl-D-glucopyranose, appeared to be somewhat effective. The effects of these compounds were, however, only moderate, and were less than those observed with some disaccharide-monoester preparations such as sucrose-mono-stearate, -palmitate, and -myristate, and related analogs of trehalose and maltose. As reported already, decrease of the dose to 50 mg/kg/day×5 did not significant-

Table I. Antitumor Effects of D-Glucosyl Monoesters upon Ehrlich Ascites Carcinoma in Mice[a]

Acyl moiety	Effect against Ehrlich ascites carcinoma					
	Monosaccharide-monoesters			Disaccharide-monoesters[b]		
	1-O-Acyl-D-glucose[c]	3-O-Acyl-D-glucose	6-O-Acyl-D-glucose	Sucrose-monoester[d]	Trehalose-monoester[e]	Maltose-monoester[f]
Capryloyl	n.d.[g]	−	−∼+	++	+	+
Lauroyl	−	−	−	++	+++	+
Myristoyl	+∼++	++[h]	−∼+	+++	+++	++
Palmitoyl	−	−	−	+++	n.d.	+++
Stearoyl	−	+[h]	++[h]	+++	+++	+++

a) Dose, 250 mg/kg/day×5; Administration route, *i.p.*
Criteria:

TPCV ratio (% treated/control)	0—10	11—40	41—65	>66
Evaluation of activity	++	++	+	−

b) Data cited from ref. 4 : some data obtained at doses other than 250 mg/kg×5 are included.
c) Anomerically pure α-anomer.
d) Isomeric mixture consisting mainly of 6-, 1′-, and 6′-monoesters.
e) Isomeric mixture consisting mainly of 6-monoester.
f) Isomeric mixture consisting mainly of 1-, 6- and 6′-monoesters.
g) n.d.: not determined.
h) The sample showed no effect at the dose of 150 mg/kg/day×5.

ly reduce the antitumor effects of disaccharide-monoesters. In contrast, complete loss of activity was observed when 3-O-myristoylglucose and 6-O-stearoylglucose were administered at the dose of 150 mg/kg/day×5. These findings suggest that monosaccharide-monoesters are less promising as antitumor drugs than the analogous derivatives of disaccharides.

The growth inhibitory effects of the glucosyl monoesters upon the cultured L-5178Y cells are shown in Table II in terms of the ID_{50} values. All the samples tested resulted in growth inhibition, at least to some extent, at the higher concentrations. However, even at the highest concentration (500 μg/ml), they failed to produce complete inhibition of the tumor growth. In a similar bioassay, sucrose-monostearate suppressed the tumor growth completely at doses of 500 and 100 μg/ml.[4c] Thus, the *in vitro* antitumor bioassay also suggests that the monoesters of monosaccharides are less effective than those of disaccharides as regards inhibition of tumor growth.

Table II. Antitumor Effects of D-Glucosyl Monoesters upon Cultured L-5178Y Cells

Acyl moiety	ID_{50} value (μg/ml)		
	1-O-Acyl-D-glucose	3-O-Acyl-D-glucose	6-O-Acyl-D-glucose
Capryloyl	281	103	147
Lauroyl	64	>500	38
Myristoyl	57	65	>500
Palmitoyl	71	>500	>500
Stearoyl	>500	444	250

The fifteen glucosyl monoesters tested gave various ID_{50} values. Relatively lower ID_{50} values were seen with the following specimens: 1-laurate, 1-myristate, 1-palmitate, 3-myristate, and 6-laurate. The *in vitro* antitumor activities of individual compounds and their *in vivo* antitumor effects described above were broadly parallel in the cases of the 1- and 3-ester series. However, in the case of the 6-ester series ,such a relationship did not seem to exist: among the 6-ester samples, the laurate, which was devoid of *in vivo* antitumor ability showed the lowest ID_{50} value, whereas the stearate, which exerted moderate *in vivo* antitumor effect, gave a rather high ID_{50} value. Accordingly, there appeared to be no direct correlation between the

antitumor results obtained in these two bioassays. In order to make a more direct comparison, we intend to carry out *in vivo* and *in vitro* bioassays using the same tumor cells in both cases.

At this time, it is not clear why the glucosyl monoesters were, in general, less effective than the analogous derivatives of disaccharides. However, this may be accounted for, presumably, by the difference in the hydrophile-lipophile-balance (HLB) between them, in view of our previous observation that the di- and higher-substituted disaccharide esters (which possessed larger HLB values) tended to have poorer antitumor effects in both bioassay systems[4]: it is apparent that the HLB values of the glucosyl monoesters are approximately equal to those of the corresponding diesters of disaccharides. Since the disaccharide-monoester preparations used in our previous antitumor tests were mixtures consisting of various positional isomers, the influence of the acyl location on the antitumor activities has not yet been elucidated. However, by analogy with our present findings, it seems probable that acyl location is an important factor for the biological activities of disaccharide-monoesters.

Acknowledgement This work was supported in part by a Grant-in-Aid from the Ministry of Health and Welfare of Japan.

References and Notes

1) Part X: Y. Nishikawa, K Yoshimoto, and M. Ohkawa, *Chem. Pharm. Bull.*, **29**, 878 (1981).
2) Presented at the 100th Annual Meeting of the Pharmaceutical Society of Japan, Tokyo, April, 1980.
3) Location: *a) 13-1 Takara-machi, Kanazawa 920, Japan*; *b) 5-1-1 Tsukiji, Chuo-ku, Tokyo 104, Japan.*
4) *a)* Y. Nishikawa, K. Yoshimoto, M. Nishijima, F. Fukuoka, and T. Ikekawa, *Chem. Pharm. Bull.*, **29**, 505 (1981); *b)* T. Ikekawa, S. Yanoma, M. Umeji, K. Yoshimoto, and Y. Nishikawa, *ibid.*, **27**, 2016 (1979); *c)* Y. Nishikawa, K. Yoshimoto, T. Manabe, and T. Ikekawa, *ibid.*, **25**, 2378 (1977); *d)* Y. Nishikawa, K. Yoshimoto, M. Okada, T. Ikekawa, N. Abiko, and F. Fukuoka, *ibid.*, **25**, 1717 (1977); *e)* Y. Nishikawa, K. Yoshimoto, M. Okabe, and F. Fukuoka, *ibid.*, **24**, 756 (1976); *f)* Y. Nishikawa, M. Okabe, K. Yoshimoto, G. Kurono, and F. Fukuoka, *ibid.*, **24**, 387 (1976).
5) *a)* Y. Nishikawa and K. Yoshimoto, *Chem. Pharm. Bull.*, **25**, 624 (1977); *b)* K. Yoshimoto, K. Tahara, S. Suzuki, K. Sasaki, Y. Nishikawa, and Y. Tsuda, *ibid.*, **27**, 2661 (1979).
6) E.N. Sassenrath, *Ann. N.Y. Acad. Sci.*, **76**, 601 (1958).
7) M. Yoshida, A. Hoshi, K. Kuretani, T. Kanai, and M. Ichino, *Gann*, **66**, 561 (1975).

[Chem. Pharm. Bull. 29(3) 883—886 (1981)]

Dissolution Properties of Salt Forms of Berberine

Shozo Miyazaki,* Midori Oshiba, and Tanekazu Nadai

Faculty of Pharmaceutical Sciences, Josai University, 1-1 Keyakidai, Sakado, Saitama, 350-02, Japan

(Received September 16, 1980)

The dissolution properties of hydrochloride, sulfate, and iodide salts of berberine were compared. The apparent solubility and dissolution rate of the hydrochloride salt were less than those of the sulfate salt in media resembling GI fluids with respect to pH. The common ion effect on the solubility of berberine hydrochloride was also investigated.

Keywords——berberine; salt form; solubility; dissolution rate; common ion effect

Salt formation is known to influence a number of physicochemical properties of the parent drug, including solubility, dissolution rate, stability, and hygroscopicity. These properties,