permethylate. It was refluxed with 2N hydrochloric acid in methanol for 4 hr and the reaction mixture was concentrated *in vacuo*, diluted with water and extracted with chloroform. The organic layer was washed with water, dried and evaporated. The residue was placed on a silica gel column and eluted with benzene–acetone (9:1, v/v). The first fraction was recrystallized from methanol to give colorless needles, mp 237°. *Anal.* Cacld. for $C_{29}H_{48}O_5$ (tokorogenin dimethylether): C, 73.07; H, 10.01. Found: C, 73.23; H, 10.08. It was identified with tokorogenin 2,3-dimethyl ether (mp 239°)[10] by mixed melting point determination and cochromatography on thin-layer of silica gel: *Rf* 0.21 (1,2-dimethyl ether[10] 0.17, 1,3-dimethyl ether[10] 0.29; solvent, hexane–ethyl acetate (4:1, v/v)). The second fraction was examined by gas liquid chromatography: $t_R$ 6.0 min (methyl 2,3,4,6-tetra-O-methyl-D-glucopyranoside 6.0 min, methyl 2,3,4,6-tetra-O-methyl-D-galactopyranoside 7.2 min; Yanagimoto Gas Chromatograph GCG-550F equipped with a hydrogen flame ionization detector, glass column, 1.2 m long, 2 mm$\phi$ packed with 5% 1,4-butanediol succinate[21] on Chromosorb W (60—80 mesh), flash heater temp. 240°, column temp. 145°, detector temp. 220°, nitrogen gas flow rate 15 ml/min).

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# *In Vivo* and *in Vitro* Antitumor Activity of Sugars containing Sulfur

Yoshinari Hasegawa, Hajime Kawasaki,[1a] Susumu Ishiguro, Takao Maki and Setsuzo Tejima[1b]

*Kyorin Chemical Laboratory[1a] and Fuculty of Pharmaceutical Sciences, Hokkaido University[1b]*

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Recently Akagi, *et al.*[2] have reported that β-D-xylopyranosyl ethylxanthate and β-D-mannopyranosyl ethylxanthate showed a marked antitumor effect on ascites type of Ehrlich carcinoma cells. This paper deals with the antitumor activity on ascitic form of Ehrlich carcinoma cells *in vivo* and Yoshida sarcoma cells *in vitro* of some new compounds.

As shown in Table I, forty compounds were screened for antitumor activity as a reference to β-D-xylopyranosyl ethylxanthate (I). The compounds I, II, X, XII, XIII, XV, XVII, XXIV, XXVI, XXVII, XXVIII and XXXVII were slightly effective on Ehrlich ascites carcinoma cells with or without toxicity to the mice. The compounds XI, XII, XIV, XVIII, XX, XXII, XXIV, XXV, XXVI, XXX, XXXI and XXXII were weakly active on Yoshida sarcoma cells *in virto*. On the contrary, the compounds I, V, IX, X, XVI and XXIII had no activity to the Yoshida sarcoma. It is interesting that the compounds I and X have been effective *in vivo* but not *in vitro*.

It is difficult to judge a parallelism between *in vivo* and *in virto*, moreover, relationship between chemical constitution and antitumor activity of thes ecompounds. However, it

1) Locat on: *a*) *1-3, Ukima, Kita-ku, Tokyo*; *b*) *Kita-15-jo, Nishi-7-chome, Sapporo.*

2) M. Akagi, S. Tejima, M. Haga, Y. Hirokawa, M. Yamada, M. Ishiguro and D. Mizuno, *Yakugaku Zasshi*, **87**, 287 (1967).

TABLE I. Antitumor Activity against Ehrlich Carcinoma Cells *in Vivo* and Yoshida Sarcoma Cells *in Vitro*

| Compound | *in Vivo* | | | *in Vitro* |
|---|---|---|---|---|
| | Dose[a] (μg/mouse/day) | Toxicity | [b] Survival day mean±S.D.[c] (No. of mice) | $IC_{50}$ (range) mM |
| β-D-Xylopyranosyl ethylxanthate (I)[2,3] | 2500 | 0/16 | 17.9±6.0 (16) | $1.2(0.3—5.2)\times10^{-2}$ |
| 2,3,4-Tri-O-acetyl-β-D-xylopyranosyl ethylxanthate (II)[3] | 2500 | 2/8 | 17.7±5.8 (6) | $1.1(0.7—1.8)\times10^{-3}$ |
| 2,3,4-Tri-O-acetyl-β-D-xylopyranosyl butylxanthate (III)[4] | 2500 | 2/8 | 9.8±0.7 (6) | $2.5(0.5—5.0)\times10^{-3}$ |
| 2,3,4-Tri-O-benzoyl-β-D-xylopyranosyl ethylxanthate (IV)[5] | 2500 | 0/8 | 12.1±0.9 (8) | $3.6(2.2—4.9)\times10^{-3}$ |
| β-D-Xylopyranosyl N,N-dimethyldithiocarbamate (V)[6] | 2000 | 5/8 | 12.3±4.7 (3) | $1.0\times10^{-1}$ |
| Methyl 1-deoxy-1-thio-2,3,4-tri-O-acetyl-β-D-xylopyranoside (VI)[5] | 2500 | 1/8 | 15.1±5.4 (7) | $4.0(3.9—4.1)\times10^{-3}$ |
| Ethyl 1-deoxy-1-thio-2,3,4-tri-O-acetyl-β-D-xylopyranoside (VII)[5] | 2500 | 2/8 | 15.7±8.1 (6) | $2.9(2.3—3.7)\times10^{-3}$ |
| Benzyl 1-deoxy-1-thio-2,3,4-tri-O-acetyl-β-D-xylopyranoside (VIII)[5] | 2500 | 1/8 | 15.9±3.4 (7) | $3.3(2.9—4.4)\times10^{-3}$ |
| Bis(β-D-xylopyranosyl)disulfide (IX)[2,4] | 2000 | 2/8 | 16.0±6.3 (6) | $1.0\times10^{-1}$ |
| 1,2-Dideoxy-1,2-dithiocarbonyl-β-D-mannopyranose (X)[5] | 1200 | 0/8 | 17.3±1.9 (8) | $4.3(3.0—6.1)\times10^{-2}$ |
| 1,2-Dideoxy-1,2-trithiocarbonyl-β-D-mannopyranose (XI)[7] | 2500 | 2/8 | 15.2±2.1 (6) | $6.4(2.2—18.9)\times10^{-4}$ |
| 1,2-Dideoxy-1,2-trithiocarbonyl-3,4,6-tri-O-acetyl-β-D-mannopyranose (XII)[7] | 2500 | 1/8 | 17.4±1.8 (7) | $1.6(0.9—2.8)\times10^{-4}$ |
| 1,2-Dideoxy-1,2-dithioimidocarbonyl-β-D-mannopyranose (XIII)[7] | 1200 | 0/8 | 18.7±4.2 (8) | $3.9(2.2—6.8)\times10^{-3}$ |
| 1,2-Dideoxy-1,2-(N-acetyl)-dithioimidocarbonyl-3,4,6-tri-O-acetyl-β-D-mannopyranose (XIV)[7] | 2500 | 0/8 | 15.4±3.1 (8) | $4.2(1.8—7.2)\times10^{-4}$ |
| 1,2-Dideoxy-1,2-(N-benzoyl)-dithioimidocarbonyl-3,4,6-tri-O-benzoyl-β-D-mannopyranose (XV)[7] | 2500 | 0/8 | 18.0±7.0 (8) | $1.4(0.6—3.6)\times10^{-3}$ |
| 1,2-Dideoxy-1,2-dithioimidocarbonyl-β-D-mannopyranose phenylhydrazone (XVI)[7] | 500 | 0/6 | 14.5±2.4 (6) | $3.0(1.5—5.8)\times10^{-2}$ |
| 1,2-Dideoxy-1,2-dithioimidocarbonyl-3,4,6-tri-O-acetyl-β-D-mannopyranose phenylhydrazone (XVII)[7] | 1200 | 0/8 | 18.9±4.5 (8) | $1.8(0.3—11.4)\times10^{-3}$ |
| 1,2-Dideoxy-1,2-(N-mesyl)dithioimidocarbonyl-3,4,6-tri-O-acetyl-β-D-mannopyranose (XVIII)[7] | 2500 | 0/8 | 16.8±5.3 (8) | $1.1(0.1—7.6)\times10^{-4}$ |
| 1,2-Dideoxy-1,2-(N-mesyl)dithioimidocarbonyl-3,4-di-O-acetyl-6-deoxy-6-iodo-β-D-mannopyranose (XIX)[7] | 1200 | 1/8 | 14.6±7.5 (7) | $1.4(0.6—3.2)\times10^{-3}$ |
| 1,2-Dideoxy-1,2-(N,N-dimethylammonium)dithiocarbonyl-β-D-mannopyranose methanesulfonate (XX)[8] | 2500 | 2/8 | 16.0±1.8 (6) | $7.8(6.4—9.4)\times10^{-4}$ |
| Methyl 1,2-dideoxy-1,2-dithio-2-S-methyl-3,4,6-tri-O-acetyl-β-D-*manno*-hexopyranoside (XXI)[5] | 2500 | 0/8 | 14.5±0.7 (8) | $3.0(2.4—3.9)\times10^{-3}$ |
| 1,2-Dideoxy-1,2-dithioacetyl-3,4,6-tri-O-acetyl-β-D-mannopyranose (XXII)[5] | 2500 | 4/8 | 15.3±8.6 (4) | $1.7(0.5—5.9)\times10^{-4}$ |
| 1,2-Dideoxy-1,2-dithioacetyl-3,4,6-tri-O-acetyl-α-D-mannopyranose (XXIII)[5] | 2500 | 1/8 | 15.6±6.0 (7) | $1.1(0.7—1.8)\times10^{-2}$ |
| 2-Deoxy-2-ethoxythiocarbonylthio-α-D-*manno*-hexopyranosyl ethylxanthate (XXIV)[5] | 2500[d] | 4/8 | 20.5±5.9 (4) | $3.3(2.2—5.0)\times10^{-4}$ |
| 2-Deoxy-2-methoxythiocarbonylthio-3,4,6-tri-O-acetyl-α-D-*manno*-hexopyranosyl methylxanthate (XXV)[5] | 1200 | 1/8 | 11.0±3.3 (7) | $9.9(5.1—19.0)\times10^{-4}$ |
| 2-Deoxy-3,4,6-tri-O-acetyl-β-D-*arabino*-hexopyranosyl N,N-diethyldithiocarbamate (XXVI)[9] | 1200 | 0/6 | 17.2±2.1 (6) | $4.1(1.1—16.1)\times10^{-4}$ |
| 3-Bromo-2,3-dideoxy-β-D-*arabino*-hexopyranosyl N,N-dimethyldithiocarbamate (XXVII)[9] | 1200 | 0/8 | 17.3±5.2 (8) | $3.6(1.8—7.6)\times10^{-3}$ |
| 3-Bromo-4,6-di-O-acetyl-2,3-dideoxy-β-D-*arabino*-hexopyranosyl N,N-dimethyldithiocarbamate (XXVIII)[9] | 2500 | 2/8 | 17.5±4.3 (6) | $4.5(2.0—9.8)\times10^{-3}$ |
| 2-Deoxy-2-N,N-dimethyldithiocarbamoyl-3,4,6-tri-O-acetyl-D-*arabino*-hexopyranose-1-ene (XXIX)[8] | 1200 | 0/8 | 14.5±0.7 (8) | $1.9(1.3—3.0)\times10^{-3}$ |
| 2-O-Mesyl-3,4,6-tri-O-acetyl-β-D-galactopyranosyl N,N-dimethyldithiocarbamate (XXX)[5] | 2500 | 1/8 | 13.0±4.6 (7) | $6.7(0.4—1.0)\times10^{-4}$ |
| 2-O-Mesyl-3,4,6-tri-O-acetyl-β-D-glucopyranosyl N,N-dimethyldithiocarbamate (XXXI)[6] | 2500 | 0/8 | 14.2±1.4 (8) | $6.6(4.5—9.9)\times10^{-4}$ |
| 2-O-Mesyl-3,4,6-tri-O-acetyl-β-D-glucopyranosyl N,N-diethyldithiocarbamate (XXXII)[8] | 2500 | 0/8 | 16.4±2.3 (8) | $7.3(5.6—9.4)\times10^{-4}$ |
| Bis(1,1′,2,2′,3,3′-hexa-O-acetyl-5,5′-dideoxy-D-xylofuranose)5,5′-sulfide (XXXIII)[5] | 2500 | 0/8 | 14.4±4.1 (8) | $2.2(1.4—3.6)\times10^{-3}$ |
| 2,3-Di-O-tosyl-5-deoxy-5-N,N-dimethyldithiocarbamoyl-DL-xylitol (XXXIV)[7] | 2500 | 1/8 | 13.4±1.7 (7) | $3.8(3.3—4.4)\times10^{-3}$ |
| Methyl 2-O-tosyl-α-D-xylofuranoside (XXXV)[10] | 2500 | 0/8 | 13.2±1.8 (8) | $3.7(2.7—4.9)\times10^{-3}$ |
| Methyl 3-O-benzyl-2,5-di-O-tosyl-α-D-xylofuranoside (XXXVI)[5] | 2500 | 0/8 | 14.2±0.9 (8) | $3.5(2.6—4.5)+10^{-3}$ |
| 3,5-Di-O-benzoyl-2-O-tosyl-β-D-xylofuranosyl isothiuronum hydrobromide (XXXVII)[5] | 1200 | 2/8 | 18.0±7.3 (6) | $1.2(1.1—1.3)\times10^{-3}$ |
| 3,5-Di-O-benzoyl-2-O-tosyl-β-D-xylofuranosyl ethylxanthate (XXXVIII)[5] | 2500 | 2/8 | 12.2±2.9 (6) | $3.9(2.3—4.7)\times10^{-3}$ |
| 2,3,5,6-Tetra-O-benzoyl-β-D-glucofuranosyl N,N-dimethyldithiocarbamate (XXXIX)[5] | 2500 | 0/8 | 14.0±1.5 (8) | $6.2(4.2—9.2)\times10^{-3}$ |
| 2,3,5,6-Tetra-O-benzoyl-β-D-glucofuranosyl methylxanthate (XXXX)[5] | 1200 | 0/8 | 15.2±1.7 (8) | $8.4(6.8—10.3)\times10^{-3}$ |

a) Administration was performed intraperitoneally once a day for 7 days, being started from the day following the inoculation with the tumor cells.
b) Deaths occuring before 9th day of tumor inoculation are considered due to toxicity.
c) The animal survived for 30 days was calculated as 30 days. Mean survival days of control group was 15.6±1.7 (31 mice).
d) Administration was stopped after 4 days performance because strong toxicity was appeared.

seems that the 2-deoxy- derivatives (XXVI, XXVII, XXVIII) have an activity against the cells *in vivo*, and the acylated compounds were more effective than the free hydroxyl compounds. The xylose derivatives contained xanthate group (I, II) have an activity only against *in vivo*. The dithioimidocarbonyl derivatives were effective on the Yoshida sarcoma but not effective on both. XXII have an effectiveness and XXIII have not. This is a compound of the $\alpha$-form on $C_{-1}$, and that is the $\beta$-form on $C_{-1}$ and in the *cis* conformation on $C_{-1}$ and $C_{-2}$ position. Furthermore, we expect to have an antitumor activity on the trithiocarbonate compounds.

## Experimental

**Synthesis of Compounds**——Compounds to be tested for the present work were synthesized by established or novel methods[3-10] on another reports.

***In Vitro* Screening Method**——Animals used were dd/Y male mice weighing approximately 24 g, and tumor cells used were Ehrlich ascites carcinoma.

The procedure used for screening was essentially the same as that described by Egashira, *et al.*[11] and Sugiura.[12] However, antitumor effect was assayed on the ascitic form only. The solution of $3\times10^6$ tumor cells in 0.2 ml physiological saline solution was inoculated intraperitoneally.

The compounds to be tested were dissolved or suspended in physiological saline solution containing 0.03% Tween 80. Administration was performed intraperitoneally once a day for 7 days, being started from the day following the inoculation with the tumor cells. The mice were observed for 30 days.

***In Vivo* Screening Method**——The cell culture was carried out as the description of Ishidate, *et al.*[13] The $6\times10^4$ Yoshida sarcoma cells which had been grown in the peritoneal cavity of Donryu female rat were cultured in 1.2 ml of the No. 18 medium containing 50% horse serum.

The compound to be tested were dissolved or suspended in physiological saline containing ethanol when was not dissolved in water, and diluted with physiological saline to give suitable volume.

The evaluation of the compounds on the Yoshida sarcoma cells *in vitro* was expressed fifty percent inhibition concentration ($IC_{50}$) as described by Moriwaki.[14]

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