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A New Class of Nitrosoureas. III.[1] Synthesis and Antitumor Activity of 3,3-Disubstituted-1-(2-chloroethyl)-1-nitrosoureas having an Arabinopyranosyl, Xylopyranosyl or Ribopyranosyl Moiety

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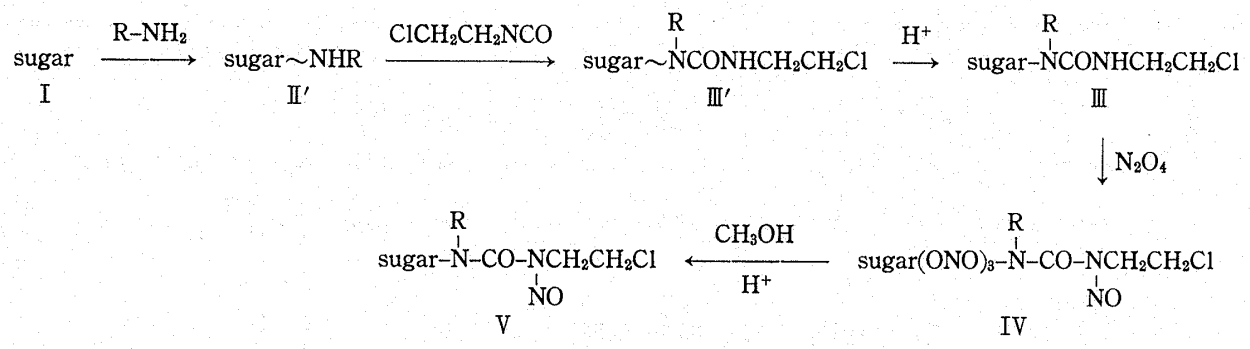
Many kinds of 3,3-disubstituted-1-(2-chloroethyl)-1-nitrosourea derivatives (V) of aldopentoses were synthesized and tested for antitumor activities against leukemia L1210 and Ehrlich ascites carcinoma. The reaction of aldopentoses with primary amines followed by treatment with 2-chloroethyl isocyanate usually gave a mixture of two or three structural isomers of glycosylureas (III'). Complete isomerization into thermodynamically stable glycopyranosylureas (III) was observed when the mixture of the isomers was dissolved in formic acid. Glycopyranosylureas (III) were nitrosated with 4 equivalents of dinitrogen tetroxide followed by treatment with methanol to give the corresponding nitrosoureas (V) in good yields. A large number of nitrosoureas (V) were remarkably active against leukemia L1210 and Ehrlich ascites carcinoma and showed greater therapeutic ratios than the positive controls, 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea, 3-[(4-amino-2-methyl-5-pyrimidinyl)methyl]-1-(2-chloroethyl)-1-nitrosourea, and 1-(2-chloroethyl)-3-(β-D-glucopyranosyl)-1-nitrosourea. In particular, many of the arabinopyranosylnitrosoureas had large therapeutic ratios (more than fifty) against leukemia L1210 and even larger ones (more than a hundred) against Ehrlich ascites carcinoma. These nitrosoureas (V) appear to be activated nonenzymatically by attack of the hydroxyl group of the sugar moiety on the carbonyl group to give the cyclic carbamate (VII) without generation of the isocyanate.

Keywords——chloroethyl nitrosoureas; 3-substituted-3-glycopyranosylnitrosoureas; antitumor activities; leukemia L1210; Ehrlich ascites carcinoma; CCNU; ACNU; GANU

Nitrosourea derivatives, including 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea (CCNU) and 3-[(4-amino-2-methyl-5-pyrimidinyl)methyl]-1-(2-chloroethyl)-1-nitrosourea (ACNU), possess a wide spectrum of antitumor activity against experimental tumors, and these compounds have been used in humans. However, delayed and cumulative damage to the bone marrow caused by these agents limits their clinical usefulness significantly.[2] Recently, water-soluble glucose-containing nitrosourea derivatives such as 2-[3-(2-chloroethyl)-3-nitrosoureido]-2-deoxy-D-glucopyranose (DCNU), 1-(2-chloroethyl)-3-(β-D-glucopyranosyl)-1-nitrosourea (GANU), *etc.*, have been reported to possess strong antitumor activity with reduced myelotoxicity.[3] In the preceding paper we reported the synthesis and antitumor activity of a new class of 3,3-disubstituted-1-(2-chloroethyl)-1-nitrosoureas having an aldohexose moiety such as D-glucose, D-mannose, or D-galactose.[1] These nitrosoureas were found to exhibit remarkable antitumor activity against leukemia L1210 and Ehrlich ascites carcinoma. In particular, some of the galactopyranosyl derivatives showed excellent antitumor activities comparable with those of active controls such as CCNU, ACNU, and GANU. The sugar moiety in these derivatives was assumed to play an important role as a carrier of the functional group. Therefore, the study was extended to the synthesis of the corresponding aldopentose derivatives. This paper describes the synthesis and the antitumor activity of a new class of 3,3-disubstituted-1-(2-chloroethyl)-1-nitrosoureas bearing an aldopentose moiety such as L- or D- arabinose, D-xylose, or D-ribose.

Synthesis of Nitrosoureas and Discussion

The aldopentose derivatives of nitrosoureas (V_{1-32}) which are disubstituted on their N-3 position were obtained by the sequence outlined in Chart 1.



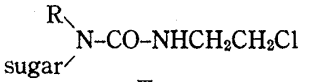
II′ and III′: represent mixtures of structural isomers

Chart 1

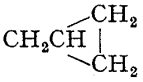
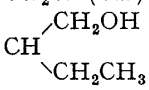
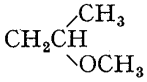
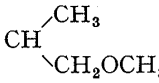
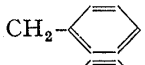
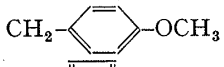
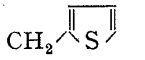
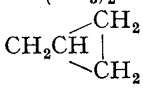
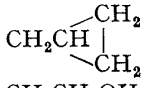
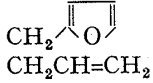
L- and D-arabinose, D-xylose, D-ribose, and 2-deoxy-D-ribose were chosen as the sugar moiety (I) in the present study. Heating of I with primary amines in methanol at 65—70°C gave a mixture of structural isomers of the *N*-substituted-glycosylamines (II′). It is known that the reaction of carbohydrates with amines frequently leads to the formation of anomeric mixtures of glycosylamines and the proportion of the isomers is strongly dependent on the reaction conditions and the type of amine.[4] The reaction of the mixture (II′) with 2-chloroethyl isocyanate usually gave the ureas (III′) as a mixture of structural isomers with respect to the sugar moiety. For instance, the reaction of L-arabinose with 3-methoxy-*n*-propylamine followed by treatment with 2-chloroethyl isocyanate gave a mixture of ureas (III'_{11}), whose thin layer chromatography (TLC) gave three spots in a ratio of 3: 2: 1 (*Rf*: 0.73, 0.68, and 0.58). In the preceding paper,[1] we reported that the complete isomerization of an isomeric mixture of the aldohexose derivatives corresponding to III′ into thermodynamically stable β-D-glycopyranosylureas occurred on treatment with formic acid. Similar isomerization was also observed in the present study. Thus, when the mixture of ureas (III'_{11}) was dissolved in a small amount of formic acid at room temperature, the three spots on TLC (*Rf*: 0.73, 0.68 and 0.58) changed completely to a spot of III_{11} (*Rf*: 0.58). III_{11} was isolated from this solution as an amorphous powder and gave infrared (IR) signals due to the ureido group at 1640 and 1550 cm^{-1} and nuclear magnetic resonance (NMR) signals due to the anomeric proton of α-L-arabinopyranoside at δ 4.90 (d, J=8 Hz). III_{11} was acetylated with acetic anhydride in pyridine to give the triacetate (VI), whose mass spectrum (MS) showed characteristic ion peaks at *m/e* 452 (M^+) and 416 ($M^+ - 36$). In contrast, III_{11} gave no tritylated product on reaction with trityl chloride in pyridine. This indicates that III_{11} has no primary hydroxyl group, because *O*-tritylation usually proceeds much more rapidly with primary hydroxyl groups than with secondary hydroxyl groups and has been used to determine quantitatively the number of primary hydroxyl groups in a sugar moiety.[5] Thus, the structure of III_{11} was determined to be 3-(α-L-arabinopyranosyl)-1-(2-chloroethyl)-3-(3-methoxy-*n*-propyl)urea (see Chart 2). The other isomers (III_{11a} and III_{11b}) with *Rf* values of 0.73 and 0.68 are assumed to be a β anomer of L-arabinopyranosylurea and an α anomer of L-arabinofuranosylurea, respectively. The details of the structures of these isomeric glycosylureas and their isomerization by acid will be reported elsewhere. Thus, a number of α-L-arabinopyranosyl, β-D-xylopyranosyl and β-D-ribopyranosylurea could easily be obtained from the mixture of structural isomers by this acid treatment. These glycopyranosylureas (III) are generally light brownish powders or caramels and are listed in Table I with some characteristic data.

The nitrosation of the ureas (III) was carried out by the use of dinitrogen tetroxide in the presence of an acid acceptor in an inert solvent at low temperature. The nitrosation of

TABLE I. Properties of Urea Derivatives of Aldopentoses



III

III No.	Sugar	R	Yield (%)	IR ν_{max}^{Nujol} cm^{-1}	NMR (in D_2O) δ
1	Arabino[a]	$CH_2CH_2CH_3$	68	3400, 1645, 1540, 1070	0.90 (3H, t), 1.40—1.90 (2H, m)
2	Arabino	$CH(CH_3)_2$	71	3350, 1660, 1540, 1090[f]	1.35 (6H, d)
3	Arabino	$CH_2CH(CH_3)_2$	73	3360, 1630, 1540, 1090[f]	0.90 (6H, d), 1.90—2.30 (1H, m)
4	Arabino		69	3350, 1640, 1535, 1060	0.35—0.75 (4H, m), 1.05—1.45 (1H, m)
5	Arabino	$CH_2CH=CH_2$	64	3340, 1630, 1530, 1080	
6	Arabino	CH_2CH_2OH	68	3400, 1640, 1550, 1080	
7	Arabino	$CH_2CH_2CH_2OH$	75	3370, 1630, 1530, 1080	1.6—2.15 (2H, m)
8	Arabino	$CH_2CH(OH)CH_3$	70	3380, 1640, 1520, 1070	1.17 (3H, d)
9	Arabino		62	3350, 1635, 1540, 1085	0.88 (3H, t)
10	Arabino	$CH_2CH_2OCH_3$	78	3350, 1640, 1540, 1090	3.35 (3H, s), 4.90 (1H, d, C_1–H)
11	Arabino	$CH_2CH_2CH_2OCH_3$	76	3370, 1640, 1530, 1090[f]	1.6—2.1 (2H, m), 3.35 (3H, s), 4.90 (1H, d, C_1–H)
12	D-Arabino[b]	$CH_2CH_2CH_2OCH_3$	73	3370, 1640, 1530, 1085[f]	1.6—2.1 (2H, m), 3.35 (3H, s), 4.90 (1H, d, C_1–H)
13	Arabino		70	3350, 1640, 1545, 1080	1.15 (3H, d), 3.38 (3H, s)
14	Arabino		64	3320, 1630, 1520, 1080	1.13 (3H, d), 3.33 (3H, d)
16	Arabino		72	3350, 1640, 1530, 1090	7.30 (5H, s)[g]
17	Arabino		74	3350, 1630, 1520, 1080	3.7 (3H, s), 7.03 (4H, ABq)[g]
18	Arabino		70	3340, 1610, 1550, 1065	7.0—7.3 (2H, m), 7.4—7.6 (1H, m)
19	Xylo[c]	$CH_2CH_2CH_3$	76	3370, 1640, 1520, 1040	0.85 (3H, t), 1.45—1.95 (2H, m)
20	Xylo	$CH(CH_3)_2$	72	3325, 1665, 1540, 1090	1.35 (6H, d)
21	Xylo		68	3350, 1640, 1530, 1050	0.2—0.68 (4H, m), 0.68—1.3 (1H, m), 4.98 (1H, d, C_1–H)
22	Xylo	CH_2CH_2OH	68	3350, 1640, 1540, 1040	
23	Xylo	$CH_2CH(OH)CH_3$	66	3350, 1630, 1530, 1040	1.16 (3H, d)
24	Xylo	$CH_2CH_2OCH_3$	75	3350, 1640, 1540, 1110, 1040	3.35 (3H, s), 5.0 (1H, d, C_1-H)
25	Ribo[d]	CH_3	78	3350, 1635, 1540, 1050	3.08 (3H, s)
26	Ribo	$CH_2CH_2CH_2CH_3$	73	3350, 1640, 1535, 1060[f]	0.7—1.9 (7H, m)
27	Ribo		70	3350, 1630, 1530, 1080[f]	0.2—0.7 (4H, m), 0.9—1.35 (1H, m)
28	Ribo	CH_2CH_2OH	74	3350, 1630, 1540, 1080	
29	Ribo	$CH_2CH_2OCH_3$	80	3320, 1630, 1540, 1110, 1080[f]	3.40 (3H, s)
30	Ribo	$CH_2CH_2OCH_2CH_3$	78	3350, 1640, 1560, 1110, 1080[f]	1.20 (3H, t)
31	Ribo		72	3400, 1650, 1520, 1080[f]	6.4 (2H, m), 7.45 (1H, m)
32	Deoxy-ribo[e]	$CH_2CH=CH_2$	70	3360, 1640, 1530, 1080[f]	1.8—2.15 (2H, m, ring protons)

a) Arabino represents an α-L-arabinopyranosyl moiety.
b) D-Arabino represents an α-D-arabinopyranosyl moiety.
c) Xylo represents a β-D-xylopyranosyl moiety.
d) Ribo represents a β-D-ribopyranosyl moiety.
e) Deoxy-ribo represents a 2-deoxy-β-D-ribopyranosyl moiety.
f) Measured in $CHCl_3$ solution.
g) Measured in d_6-DMSO–D_2O.

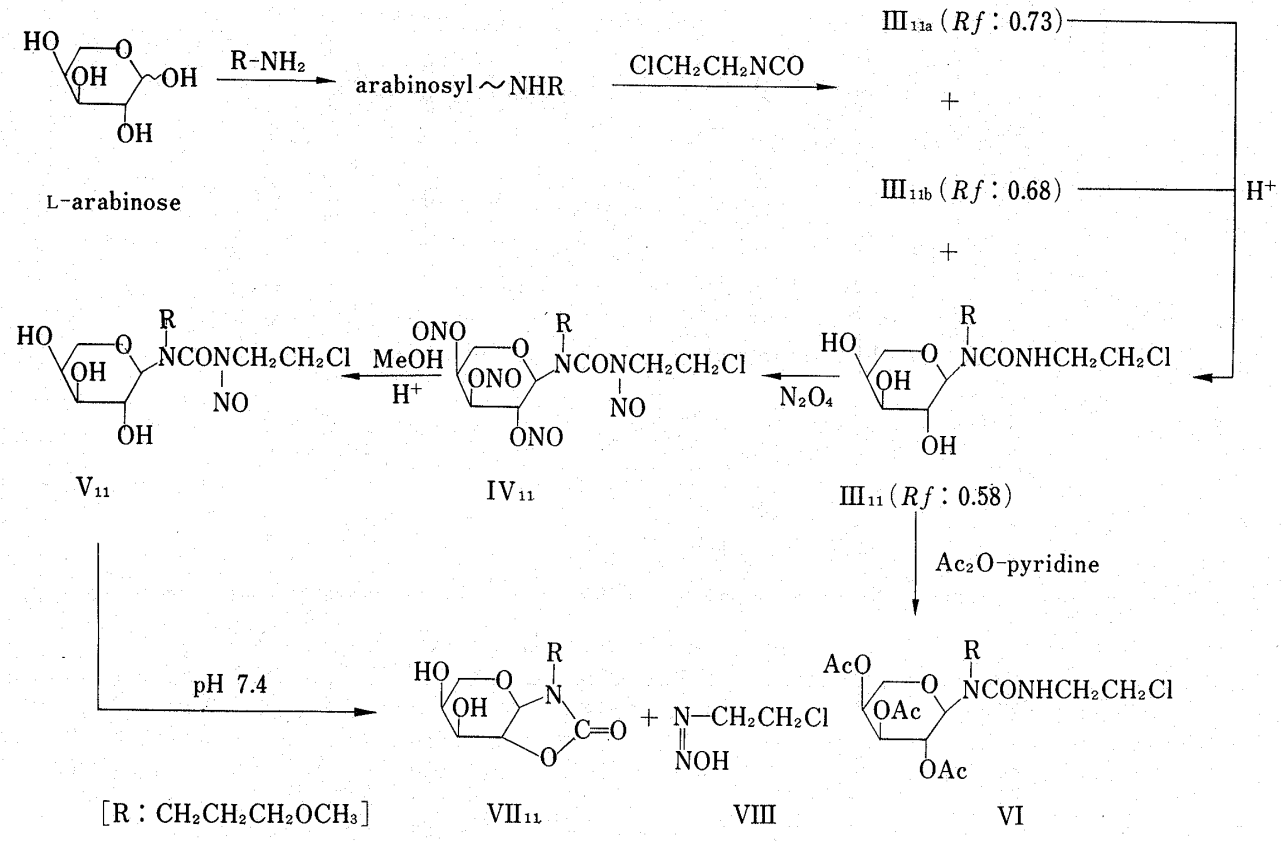


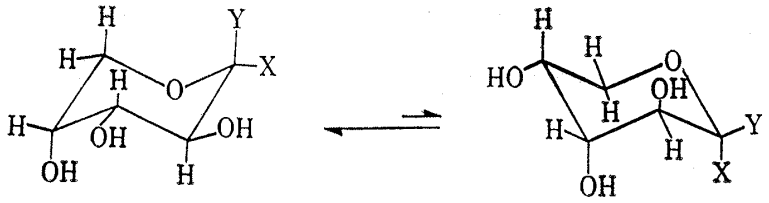
Chart 2

III required four equivalents of dinitrogen tetroxide, indicating that the three hydroxyl groups in a glycopyranosyl moiety and one ureido group are nitrosated. The nitrous esters of the hydroxyl groups could be decomposed without affecting the *N*-nitroso group by the addition of methanol under acidic conditions at low temperature. Thus, in a typical procedure, four equivalents of dinitrogen tetroxide was introduced into a mixture of the urea (III_{11}) and anhydrous sodium acetate in tetrahydrofuran at −5°C to form the nitrous ester (IV_{11}) quantitatively. Methanol was added at the same temperature and the mixture was extracted with ethyl acetate after addition of sodium acetate and a small amount of water. The nitrosourea (V_{11}) was obtained in 69% yield as yellow crystals, mp (dec.) 101—102°C, $[\alpha]_D^{22}$ +50.0° (c=1.6, methanol). V_{11} showed the IR signal due to the nitrosoureido group at 1700 cm^{-1} and the NMR signal due to the anomeric proton of α-L-arabinopyranoside at δ 4.90 (d, J=8 Hz). Thus, the structure of V_{11} was confirmed to be 3-(α-L-arabinopyranosyl)-1-(2-chloroethyl)-3-(3-methoxy-*n*-propyl)-1-nitrosourea. On the other hand, 3-(α-D-arabinopyranosyl)-1-(2-chloroethyl)-3-(3-methoxy-*n*-propyl)-1-nitrosourea (V_{12}) was similarly obtained by starting from D-arabinose and 3-methoxy-*n*-propylamine, mp 101—102°C, $[\alpha]_D^{22}$ −50.1° (c=1.4, methanol). V_{12} is the enantiomer of V_{11} and gave an IR band at 1700 cm^{-1} and an NMR signal due to the anomeric proton of α-D-arabinopyranoside at δ 4.90 (d, J=8 Hz).

In the sugar of D-series, the stable conformer usually has the C1 conformation and hence the β isomer is thermodynamically more stable than the α isomer.[6] However, in the case of D-arabinose, the stable conformer is known to have the 1C conformation and therefore the α-isomer is thermodynamically more stable than the β-isomer[6] (see Fig. 1).

It is unusual that V_{11} and V_{12} are well-difined crystalline compounds, because 3,3-disubstituted-1-nitrosourea derivatives are generally obtained as amorphous powders or caramels.

Thus, a large number of 3-substituted-1-(2-chloroethyl)-3-glycopyranosyl-1-nitrosoureas (V) were obtained in this way. These nitrosoureas (V) were generally unstable yellow caramels

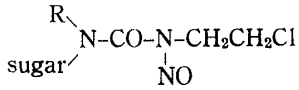


1C Cl

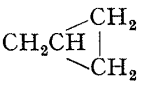
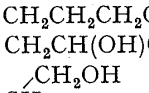
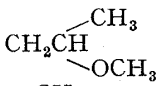
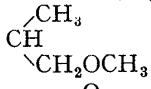
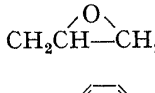
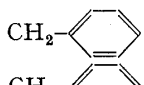
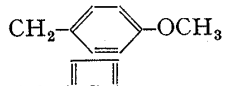
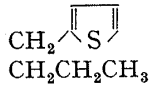
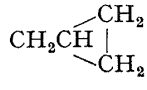
α: X=ureido, Y=H
β: X=H, Y=ureido

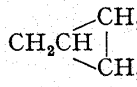
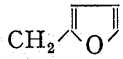
Fig. 1. Chair Conformers of D-Arabinopyranosyl Derivatives

TABLE II. Properties of Nitrosourea Derivatives of Aldopentoses



V

$V_{No.}$	Sugar	R	Yield (%)	$[\alpha]_D^{a)}$	IR $\nu_{max}^{CHCl_3}$ (cm^{-1})	NMR (in D_2O) δ
1	Arabino[b]	$CH_2CH_2CH_3$	68	+44.5°	3400, 1695, 1080	0.9 (3H, t), 1.4—1.9 (2H, m)
2	Arabino	$CH(CH_3)_2$	66	+64.4°	3400, 1695, 1080	1.35 (6H, d), 4.15 (2H, t)[j]
3	Arabino	$CH_2CH(CH_3)_2$	71	+28.0°	3400, 1690, 1080	0.95 (6H, d), 1.9—2.4 (1H, m), 4.10 (2H, t)[j]
4	Arabino		73	+54.7°	3400, 1700, 1090	0.2—0.8 (4H, m), 0.9—1.4 (1H, m)
5	Arabino	$CH_2CH=CH_2$	65	+12.8°	3400, 1700, 1080	
6	Arabino	CH_2CH_2OH	60	+40.2°	3400, 1700, 1070[g]	
7	Arabino	$CH_2CH_2CH_2OH$	63	+48.2°	3400, 1695, 1080[g]	1.8—2.25 (2H, m)
8	Arabino	$CH_2CH(OH)CH_3$	58	+39.1°	3380, 1695, 1080[g]	
9	Arabino		59	+5.8°	3380, 1680, 1080[g]	0.85 (3H, t), 1.4—2.0 (2H, m)
10	Arabino	$CH_2CH_2OCH_3$	70	+45.5°	3420, 1695, 1080	3.35 (3H, s), 4.2 (2H, t),[j] 4.90 (1H, d, C_1–H)
11	Arabino	$CH_2CH_2CH_2OCH_3$	69	+50.0°	3400, 1700, 1075[g]	1.75—2.2 (2H, m), 3.35 (3H, s), 4.10 (2H, t),[j] 4.90 (1H, d, C_1–H)
12	D-Arabino[c]	$CH_2CH_2CH_2OCH_3$	73	−50.1°	3400, 1700, 1075[g]	1.75—2.2 (2H, m), 3.33 (3H, s), 4.16 (2H, t),[j] 4.83 (1H, d, C_1–H)[h]
13	Arabino		72	+30.5°	3400, 1695, 1080	1.20 (3H, d), 3.34 (3H, s)
14	Arabino		67	+42.6°	3400, 1700, 1080	1.40 (3H, d), 3.30 (3H, s)
15	Arabino		16[i]	+38.4°	3420, 1700, 1080	2.7—3.05 (3H, m), 4.20 (2H, t)[j]
16	Arabino		72	−15.6°	3400, 1695, 1070[g]	4.70 (2H, s), 4.86 (1H, d, C_1–H), 7.30 (5H, s)[h]
17	Arabino		76	−16.6°	3400, 1695, 1080[g]	3.7 (3H, s), 4.6 (2H, s), 4.8 (1H, d, C_1–H), 7.03 (4H, ABq)
18	Arabino		74	+14.1°	3400, 1700, 1080	7.0—7.3 (2H, m), 7.4—7.6 (1H, m)
19	Xylo[d]	$CH_2CH_2CH_3$	72	+5.8°	3400, 1695, 1070	0.85 (3H, t), 1.4—1.9 (2H, m), 4.15 (2H, t)[j]
20	Xylo	$CH(CH_3)_2$	70	+22.2°	3400, 1700, 1075	1.35 (6H, d), 4.15 (2H, t)[j]
21	Xylo		68	+11.0°	3400, 1700, 1080	0.2—0.7 (4H, m), 0.94—1.4 (1H, m), 4.18 (2H, t)[j]

$V_{No.}$	Sugar	R	Yield (%)	$[\alpha]_D$[a]	IR $\nu_{max}^{CHCl_3}$ (cm^{-1})	NMR (in D_2O) δ
22	Xylo	CH_2CH_2OH	56	+4.3°	3350, 1700, 1050	
23	Xylo	$CH_2CH(OH)CH_3$	58	+1.4°	3400, 1700, 1070	1.2 (3H, d), 4.2 (2H, t)[j]
24	Xylo	$CH_2CH_2OCH_3$	74	+4.9°	3410, 1695, 1080	3.35 (3H, s)
25	Ribo[e]	CH_3	75	−35.2°	3400, 1690, 1060	3.10 (3H, s), 5.30 (1H, d, C_1–H)
26	Ribo	$CH_2CH_2CH_2CH_3$	72	−3.0°	3400, 1700, 1080	0.7—2.0 (7H, m), 5.20 (1H, d, C_1–H)
27	Ribo		70	−8.2°	3450, 1700, 1080	0.2—0.7 (4H, m), 1.0—1.4 (1H, m)
28	Ribo	CH_2CH_2OH	60	+8.7°	3400, 1690, 1080[g]	
29	Ribo	$CH_2CH_2OCH_3$	74	−8.2°	3425, 1710, 1080	3.36 (3H, s), 4.24 (2H, t)[j]
30	Ribo	$CH_2CH_2OCH_2CH_3$	76	−17.1°	3420, 1700, 1080	1.16 (3H, t), 4.20 (2H, t)[j]
31	Ribo		71	−2.8°	3450, 1700, 1070[g]	6.39 (2H, m), 7.44 (1H, m)
32	Deoxy-ribo[f]	$CH_2CH=CH_2$	78	−50.9°	3400, 1700, 1080	1.9—2.3 (2H, m), 4.25 (2H, t)[j]

a) Measured in methanol at 15—25°C.
b) Arabino represents an α-L-arabinopyranosyl moiety.
c) D-Arabino represents an α-D-arabinopyranosyl moiety.
d) Xylo represents a β-D-xylopyranosyl moiety.
e) Ribo represents a β-D-ribopyranosyl moiety.
f) Deoxy-ribo represents a 2-deoxy-β-D-ribopyranosyl moiety.
g) Measured in Nujol mull.
h) Measured in d_6-DMSO–D_2O.
i) V_{15} was prepared by epoxidation of V_5 with *m*-chloroperbenzoic acid (see experimental section).
j) Signals due to $N(NO)C\underline{H}_2CH_2Cl$.

except for some of the β-D-ribopyranosyl derivatives which were unstable yellow oils. They are listed in Table II with some characteristic data.

Meanwhile, in the preceding paper[1] we reported the activation mechanism of 3,3-disubstituted-1-nitrosoureas having a glycopyranosyl moiety. We suggested that these nitrosoureas would be activated nonenzymatically by attack of the hydroxyl group at the C-2 position of a glycopyranosyl moiety on the carbonyl group to give the cyclic carbamate (VII) and the chloroethyl diazohydroxide (VIII) or a related compound without generation of the isocyanate (IX) (see Chart 3). This activation mechanism differs distinctly from that of known nitrosoureas such as BCNU and CCNU, which are activated by loss of the proton on the N-3 position to give VIII and IX.[7] A similar activation mechanism was expected for the aldopentose derivatives prepared in the present study.

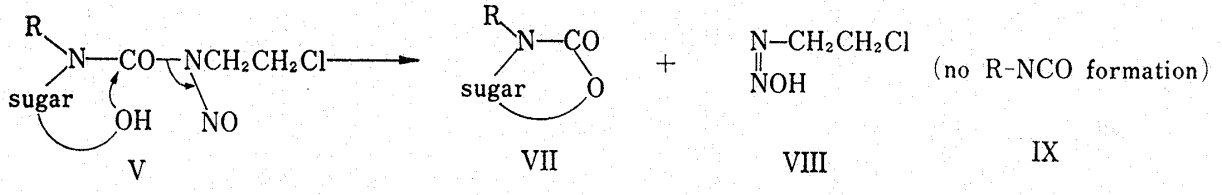


Chart 3

In fact, the nitrosourea (V_{11}) readily decomposed in phosphate-buffered solution (pH 7.4) to give colorless crystals (VII_{11}), mp 109—110°C, in high yield. VII_{11} showed the characteristic IR absorption band at 1775 cm^{-1} due to a five-membered cyclic carbamate and NMR signals due to the 3-methoxy-*n*-propyl group at δ 1.74 and 3.21, as well as that of the anomeric proton of α-L-arabinopyranoside at δ 4.36 (d, J=7.6 Hz). The mass spectrum of VII_{11} exhibited characteristic peaks at *m/e* 232 (M^+ −15 (CH_3)), 202 (M^+ −45 (COOH)), 188 (M^+ −59 ($CH_2CH_2OCH_3$)), and 160 (M^+ −87 ($NCH_2CH_2CH_2OCH_3$)). The structure of VII_{11} was thus determined to be 1-(3-methoxy-*n*-propyl)amino-1-deoxy-α-L-arabinopyranos-1,2-carbamate.

Antitumor Activities of the Nitrosoureas (V_{1-32}) and Discussion

The glycopyranosylnitrosoureas (V_{1-32}) were tested for antitumor activities against leukemia L1210 and Ehrlich ascites carcinoma by the methods described in the preceding paper.[1] The results are summarized in Table III together with comparative data for positive controls, CCNU, ACNU, and GANU.

A large number of the nitrosoureas prepared in the present study were remarkably active against both leukemia L1210 and Ehrlich ascites carcinoma and showed greater therapeutic

Table III. Antitumor Activities of Nitrosourea Derivatives of Aldopentoses (V)

$V_{No.}$	Anti-L1210 activity[a]				Anti-Ehrlich activity[b]		
	ILS_{30}[c] (mg/kg/d×5)	OD[d] (mg/kg/d×5)	ILS_{max} (%)	Therapeutic[e] ratio	MED[f] (mg/kg/d×5)	MTD[g] (mg/kg/d×5)	Therapeutic[h] ratio
CCNU	4.9	25	>757.1[i]	5.1	12.5	50	4
ACNU	2.90	25	>757.1[i]	9.3	3.12	25	8
GANU	0.80	6.25	>198.6	7.8	0.39	12.5	32
1	1.50	50	>710.8[i]	33.3	0.78	100	128
2	0.78	25	>757.7[i]	32.1	0.78	50	64
3	1.80	50	>710.8[i]	27.8	0.78	50	64
4	1.10	50	>721.9[i]	45.5	0.78	100	128
5	1.30	50	>700.0[i]	38.5	0.78	100	128
6	6.6	100	104.3	15.2	12.5	200	16
7	2.05	50	>640.7[i]	24.4	1.56	100	64
8	2.40	100	138.3	41.7	12.5	200	16
9	0.78	50	>721.9[i]	64.1	0.78	100	128
10	0.77	50	>733.3[i]	64.6	0.39	50	128
11	0.96	50	>650.0[i]	52.1	0.39	50	128
12	0.87	50	>640.7[i]	57.5	0.78	100	128
13	0.50	25	>733.3[i]	50.0	0.39	50	128
14	0.43	25	>721.9[i]	58.1	0.39	50	128
15	1.05	25	>679.2[i]	23.8	0.39	50	128
16	1.6	50	>594.4	31.3	0.78	100	128
17	1.35	50	>438.9	37.0	1.56	100	64
18	0.7	50	>315.6	71.4	0.78	100	128
19	2.0	50	>622.9[i]	25.0	0.78	50	64
20	0.65	25	>757.1[i]	38.5	0.39	25	64
21	0.95	25	>733.3[i]	26.3	0.78	25	32
22	4.1	100	82.9	24.4	6.25	100	16
23	2.5	100	>243.8	40.0	6.25	200	32
24	0.65	25	>733.3[i]	38.5	0.39	25	64
25	3.8	50	>611.3	13.2	3.12	200	64
26	7.8	100	>614.3[i]	12.8	3.12	100	32
27	1.2	25	>757.1[i]	20.8	3.12	50	16
28	2.05	50	>315.8	24.4	3.12	100	32
29	0.87	50	>757.1[i]	57.5	0.78	50	64
30	1.60	50	>733.3[i]	31.3	0.78	50	64
31	2.80	100	>434.7	35.7	1.56	100	64
32	50	200	94.4	4.0	25	200	8

a) Leukemic cells (10^5) were inoculated *i.p.* into male BDF_1 mice; *i.p.* drug administration was begun 24 h after the inoculation and performed once daily for 5 d.
b) The ascites cells (10^6) were inoculated *i.p.* into female ICR mice; *i.p.* drug administration was begun 24 h after the inoculation and performed once daily for 5 d.
c) Daily dose providing a 30% increase in life-span over the control. $ILS(\%)=(T/C-1)\times100$.
d) Optimal dose: the daily dose providing the maximum increase in life-span.
e) Therapeutic ratio=OD/ILS_{30}.
f) Minimum effective dose: the minimum dose which shows 100% inhibition of the growth of the tumor.
g) Maximum tolerated dose: the maximum dose which shows 100% inhibition of the growth of the tumor without causing the death of mice.
h) Therapeutic ratio=MTD/MED.
i) All treated mice survived for more than sixty days.

ratios than the three positive controls. Sixty-day survivors with leukemia L1210 (an indicator of complete cure) were found at the optimal dose for many of these nitrosoureas.

Meanwhile, in the preceding paper we suggested that the sugar moiety of nitrosoureas might act as a specific carrier to transport the functional group into tumor cells, and that a galactopyranosyl moiety might be the most favorable sugar moiety among aldohexoses. In the present study, as can be seen in Table III, an arabinopyranosyl moiety appears to be the most favorable sugar moiety among aldopentoses including L- and D-arabinose, D-xylose, and D-ribose. However, there is no difference in antitumor activities between L- and D-arabinopyranosylnitrosourea derivatives (see V_{11} and V_{12}). However, it is worth noting that the antitumor activity of the 2-deoxy-β-D-ribopyranosylnitrosourea derivative (V_{32}) was greatly inferior to those of other nitrosourea derivatives. This endorses the apparent significance of a hydroxyl group at the C-2 position of the sugar moiety for high antitumor activity in this class of compounds in view of the activation mechanism discussed above. On the other hand, another substituent (R) on the N-3 position also plays an important role in the antitumor activities of these nitrosoureas. Optimal activity is found in the compounds bearing alkoxyalkyl groups such as 2-methoxy-ethyl (V_{10}, V_{24} and V_{29}), 3-methoxy-propyl (V_{11} and V_{12}), 2-ethoxy-ethyl (V_{30}), and 2-methoxy-(1 or 2-methyl)-ethyl groups (V_{13} and V_{14}). As a consequence of combining the effects of a suitable sugar moiety and R group, many arabinopyranosylnitrosoureas (V_{10-14}) showed excellent antitumor activities and had large therapeutic ratios (more than fifty against leukemia L1210 and ones more than a hundred against Ehrlich ascites carcinoma). Other compounds (V_9 and V_{29}) also showed high antitumor activities.

Further studies on the synthesis and antitumor activity of this new class of nitrosoureas bearing other sugar moieties are in progress.

Experimental

IR spectra were determined with a Hitachi IR-215 spectrometer in Nujol mull or in chloroform solution. NMR spectra were recorded with a JEOL-PMX 60 spectrometer using tetramethylsilane as an internal standard in d_6-DMSO–D_2O and in $CDCl_3$, or using sodium 3-(trimethylsilyl)propionic acid in D_2O. The optical rotations were measured ina 0.5-dm tube with a Jasco DIP-180 polarimeter. Column chromatography was carried out by the use of Merck silica gel 60. TLC was performed on Merck silica gel 60 F254 TLC plates and 30% sulfuric acid was used as the spray reagent.

General Procedure for the Preparation of Glycosylamine(II'_{1-32})——A mixture of sugar (0.01 mol), amine (0.012—0.015 mol), and 10 ml of methanol was stirred and heated at 65°C for 20—40 min. The reaction mixture was concentrated to dryness under reduced pressure and ethanol was added to the residue. The mixture was again concentrated to dryness and the residue was washed with ether. In general, a mixture of structural isomers of glycosylamines was obtained as an amorphous powder or caramel in nearly quantitative yield and was used in the next step without any purification.

General Procedure for the Preparation of 3-Substituted-1-(2-chloroethyl)-3-glycopyranosylureas (III_{1-32})——Glycosylamine (0.01 mol) was dissolved in 30 ml of methanol. A solution of 2-chloroethyl isocyanate (0.012—0.015 mol) in 5 ml of tetrahydrofuran was added dropwise to the above solution at 5°C and then the mixture was stirred for 1.5 h at room temperature and concentrated under reduced pressure. The residue showed two or three spots due to the presence of the structural isomers of glycosylureas on TLC. The mixture of urea was dissolved in 10 ml of formic acid and the solution was allowed to stand at room temperature for 10 min. The TLC of the solution gave only one spot, ascribable to the thermodynamically stable glycopyranosylurea. Next 100 ml of a mixture of *n*-hexane–ethyl ether (2: 1) was added to the solution and the mixture was stirred. The oil that separated was collected and washed with ethyl ether. The crude product was chromatographed on silica gel (solvent: AcOEt–benzene–MeOH). The ureas obtained were generally light brownish powders or caramels and are listed in Table I with some characteristic data. The ureas (III_{30} and III_{32}) were pale yellow oils. The urea (III_{22}) was isomerized by using acetic acid instead of formic acid because of its instability.

Preparation of 3-(2,3,4-Tri-*O*-acetyl-α-L-arabinopyranosyl)-1-(2-chloroethyl)-3-(3-methoxypropyl)urea (VI)——A mixture of III_{11} (1.63 g, 0.005 mol), acetic anhydride (7 ml), and pyridine (15 ml) was stirred at room temperature overnight. The reaction mixture was poured into water and extracted with ethyl acetate. The organic layer was washed with cold aqueous hydrochloric acid, water, aqueous sodium bicarbonate, and aqueous sodium chloride successively. The organic layer was dried over $MgSO_4$ and concen-

trated. The residue was chromatographed on a silica gel to give the triacetate (VI) of III_{11} in 75% yield as a colorless caramel. $[\alpha]_D^{20}$ −35.8° (c=2.4, methanol). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3380, 1750, 1655, 1535, 1240, 1095, 1060, 1025. MS m/e: 452 (M^+, Calcd for $C_{18}H_{29}ClN_2O_9$), 416 (M^+−HCl). NMR (in $CDCl_3$) δ: 2.00 (6H, s, (eq-$CH_3CO)_2$), 2.16 (3H, s, ax-CH_3CO), 3.36 (3H, s, OCH_3), 6.24 (1H, br t, NH).

General Procedure for the Preparation of 3-Substituted-1-(2-chloroethyl)-3-glycopyranosyl-1-nitrosoureas (V_{1-32})——The glycopyranosylurea (III) (0.01 mol) was dissolved in 40 ml of tetrahydrofuran and then anhydrous sodium acetate (0.04 mol) was added. Dinitrogen tetroxide (0.045 mol) was introduced into the mixture at −5°C for 10 min under vigorous stirring. After 10 min 7 ml of methanol was added to the mixture and the whole was stirred at the same temperature for 10 min. Cold ethyl acetate (40 ml), anhydrous sodium acetate (0.03 mol), and 8 ml of water were then added at −5°C under stirring. The mixture was stirred vigorously for 10 min and the pH of the mixture was confirmed to be about 5. After filtration, the organic layer was collected, dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The residue was purified by silica gel chromatography (solvent: AcOEt–benzene–MeOH). The nitrosoureas (V_{1-32}) thus obtained were usually unstable yellow caramels and are listed in Table II together with the yields and some characteristic data. The following compounds had melting (decomposition) points, mp (dec.) °C: V_7, 56; V_8, 61; V_9, 53; V_{11}, 101—102; V_{12}, 101—102; V_{13}, 60; V_{18}, 63—70; V_{25}, 57—60; V_{31}, 67. Other compounds (V_{29}, V_{30} and V_{32}) were yellow oils. V_8, *Anal.* Calcd for $C_{11}H_{20}ClN_3O_7$: C, 38.65; H, 5.89; Cl, 10.37; N, 12.29. Found: C, 38.81; H, 5.84; Cl, 9.94; N, 11.82. V_{11}, *Anal.* Calcd for $C_{12}H_{22}ClN_3O_7$: C, 40.51; H, 6.23; Cl, 9.96; N, 11.81. Found: C, 40.39; H, 6.17; Cl, 10.06; N, 11.74. V_{12}, *Anal.* Calcd for $C_{12}H_{22}ClN_3O_7$: C, 40.51; H, 6.23; Cl, 9.96; N, 11.81. Found: C, 40.43; H, 6.28; Cl, 9.86: N, 11.67. V_{18}, *Anal.* Calcd for $C_{13}H_{18}ClN_3O_6S$: C, 41.10; H, 4.77; Cl, 9.33; N, 11.06; S, 8.44. Found: C, 41.28; H, 4.89; Cl, 8.89; N, 10.49; S, 8.06. V_{25}, *Anal.* Calcd for $C_9H_{16}ClN_3O_6$: C, 36.31; H, 5.41; Cl, 11.90; N, 14.11. Found: C, 36.46; H, 5.49; Cl, 11.71; N, 13.78. The nitrosourea (V_{15}) was obtained by the epoxidation of V_5 with *m*-chloroperbenzoic acid as described below.

Preparation of 3-(α-L-Arabinopyranosyl)-1-(2-chloroethyl)-1-nitroso-3-(oxyran-2-yl-methyl)urea (V_{15})——A mixture of V_5 (4.85 g, 0.015 mol), *m*-chloroperbenzoic acid (5.18 g, 0.03 mol), benzene (50 ml), and dichloromethane (50 ml) was stirred at room temperature for 3 h then concentrated under reduced pressure. The residue was washed with ethyl ether and chromatographed on silica gel (solvent: AcOEt–benzene–MeOH=10: 4: 1). The nitrosourea (V_{15}) was obtained as a yellow caramel in 16% yield. The *Rf* value of V_{15} was 0.27, while that of V_5 was 0.40 (solvent: AcOEt–chloroform–MeOH=10: 4: 1). The physical data for V_{15} are listed in Table II.

Decomposition of the Glycopyranosylnitrosourea (V_{11}) in Phosphate-buffered Solution (pH 7.4)——The nitrosourea (V_{11}) (1.0 g) was dissolved in 30 ml of 1 M phosphate buffered solution (pH 7.4) at 5°C and the mixture was stirred for 30 min. Then the solution was allowed to stand at room temperature for 20 h. The mixture was saturated with ammonium sulfate and extracted twice with a mixture of ethyl acetate and tetrahydrofuran (1: 4). The organic layer was dried over $MgSO_4$ and concentrated. The residual colorless caramel, which gave only a single spot on TLC, was purified by short column chromatography using silica gel to give 1-(3-methoxy-*n*-propyl)amino-1-deoxy-α-L-arabinopyranos-1,2-carbamate (VII_{11}) in 81% yield as colorless crystals, mp 109—110°C (from ethyl acetate). $[\alpha]_D^{20}$ +53.6° (c=1.2, methanol). IR ν_{max}^{Nujol} cm^{-1}: 3420, 1775, 1095, 1045, 1030. MS m/e: 232 (M^+-CH_3), 202 ($M^+-CH_3OCH_2$), 188 ($M^+-CH_3OCH_2CH_2$), 160 ($M^+-CH_3OCH_2CH_2CH_2N$), 116 ($M^+-CH_3OCH_2CH_2CH_2N$, COO). NMR (in d_6-DMSO–D_2O) δ: 1.74 (2H, m, $CH_2\underline{CH_2}CH_2$), 3.21 (3H, s, OCH_3), 4.36 (1H, d, J=7.6 Hz, H-1). *Anal.* Calcd for $C_{10}H_{17}NO_6$: C, 48.58; H, 6.88; N, 5.67. Found: C, 48.49; H, 6.84; N, 5.76.

Preparation of Active Controls, CCNU, ACNU and GANU——These active controls were synthesized in our laboratory as reported in the preceding paper.[1]

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