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A New Class of Nitrosoureas. V.[1] Structure of Isomeric L-Arabinosylureas and Isomerization Thereof

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Structural determination of the isomeric L-arabinosylureas (**1a**, **2a**, and **3a**) and their facile isomerization into the thermodynamically stable isomer (**3a**) by acid are described. The reaction of L-arabinose with 3-methoxy-*n*-propylamine followed by treatment with 2-chloroethyl isocyanate gave a mixture of the ureas (**1a**, **2a**, and **3a**) in a ratio of 3: 2: 1. They were determined to be the β anomer (**1a**) and the α anomer (**3a**) of 3-(L-arabinopyranosyl)-1-(2-chloroethyl)-3-(3-methoxy-*n*-propyl)urea and the β anomer (**2a**) of the corresponding L-arabinofuranosyl derivative, respectively. Isomerization of these ureas was examined in formic acid solution by monitoring their thin layer chromatography. Complete isomerization of both **1a** and **2a** into **3a** was observed, while **1a** isomerized into **3a** *via* **2a**. Consequently, the selective isomerization of a mixture of these ureas into **3a** could readily be effected by formic acid treatment. The nitrosation of these ureas by the use of dinitrogen tetroxide gave the corresponding nitrosoureas (**4a**, **5a**, and **6a**), and their structures are discussed.

Keywords——isomers of L-arabinosylurea; structural determination; isomerization by acid; isomers of L-arabinosylnitrosourea

In the previous paper,[2] we reported the synthesis of a series of 3-substituted-1-(2-chloroethyl)-3-glycopyranosyl-1-nitrosoureas which showed excellent antitumor activity against leukemia L1210 and Ehrlich ascites carcinoma. During the course of this study, we found that the reaction of sugars with primary amines followed by treatment with 2-chloroethyl isocyanate produces an isomeric mixture of glycosylureas which, on treatment with acid, readily undergoes isomerization to a thermodynamically stable isomer. Since the structures of these isomers were not established, the detailed process of this isomerization remained unkown. The present paper deals with the structures of the isomeric arabinosylureas (**1a**—**3a**) and their facile isomerization by acid as a representative example of the isomerization of many kinds of isomeric aldopentosylureas.

The reaction of L-arabinose with 3-methoxy-*n*-propylamine followed by treatment with 2-chloroethyl isocyanate gave a mixture of ureas whose thin layer chromatography (TLC) showed three spots in a ratio of 3: 2: 1 (*Rf*: 0.73, 0.68, and 0.58) (see Fig. 2, A). This mixture was separated by column chromatography on silica gel to give the ureas, **1a** (*Rf*: 0.78), **2a** (*Rf*: 0.68), and **3a** (*Rf*: 0.58). These ureas, which showed similar IR absorption bands, were acetylated by acetic anhydride in pyridine to give the corresponding acetates (**1b**, **2b**, and **3b**). The mass spectra of these acetates showed the same molecular ion peak at *m/e* 452 (see Table I). Consequently, the ureas (**1a**, **2a**, and **3a**) were considered to be structural isomers with respect to a sugar moiety. On treatment of these ureas with trityl chloride in pyridine, only **2a** gave the tritylated product (**2c**). This indicates that **2a** has a furanoid structure possessing one primary hydroxyl group, while **1a** and **3a** have a pyranoid ring structure. This was also supported by the mass spectra of the acetates (**1b**, **2b**, and **3b**) in which only **2b** showed a characteristicion peak at *m/e* 379 ($M^+ - CH_2OAc$) (see Table I).

The configurations at C-1 of these ureas were determined from their nuclear magnetic resonance (NMR) spectral data. The spectra of **1a** and **1b** showed the anomeric protons at δ 5.35 (d, J=1.5 Hz) and δ 5.77 (d, J=2.0 Hz), respectively, indicating that they are

Table I. Mass Spectral Data for Urea and Nitrosourea Derivatives of L-Arabinose

Compound No. (Formula)	*m/e*	Compound No. (Formula)	*m/e*
1b	452 (M^+)	**4b**	481 (M^+)
($C_{18}H_{29}ClN_2O_9$=452)	416 (M^+−HCl)	($C_{18}H_{28}ClN_3O_{10}$=481)	451 (M^+−NO)
	392 (M^+−AcOH)		259 (M^+−S)[a]
2b	452 (M^+)	**5b**	481 (M^+)
($C_{18}H_{29}ClN_2O_9$=452)	416 (M^+−HCl)	($C_{18}H_{28}ClN_3O_{10}$=481)	451 (M^+−NO)
	379 (M^+−CH_2OAc)		378 (M^+−NO, CH_2OAc)
2c	568 (M^+)	**5c**	517 (M^+−T)[b]
($C_{31}H_{37}ClN_2O_6$=568)	532 (M^+−HCl)	($C_{31}H_{36}ClN_3O_7$=597)	489 (M^+−U)[c]
	295 (M^+−CH_2OCPh_3)	**6b**	481 (M^+)
3b	452 (M^+)	($C_{18}H_{28}ClN_3O_{10}$=481)	451 (M^+−NO)
($C_{18}H_{29}ClN_2O_9$=452)	416 (M^+−HCl)		259 (M^+−S)[a]
	392 (M^+−AcOH)		

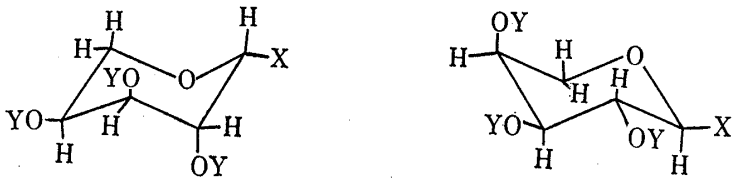
$CH_2CH_2CH_2OCH_3$

a) S=N–CO–N(NO)–CH_2CH_2Cl.
b) T=CH_3, NO, Cl.
c) U=N(NOH)CH_2CH_2Cl.

Table II. NMR Data for Urea and Nitrosourea Derivatives of L-Arabinose

Compound No.	H-1 (1H, d)	CH_3CO (s) and C_6H_5 (s)
1a[a]	5.35 (J=1.5 Hz)	
1b[b]	5.77 (J=2.0 Hz)	2.00 (3H, eq-AC), 2.15 (6H, ax-Ac)
2a[a]	5.37 (J=6.4 Hz)	
2b[b]	5.92 (J=5.2 Hz)	2.09 (9H, Ac)
2c[a]	5.51 (J=6.0 Hz)	7.35 (15H, C_6H_5)
3a[a]	4.67 (J=8.1 Hz)	
3b[b]	not assigned	2.00 (6H, eq-Ac), 2.16 (3H, ax-Ac)
4b[b]	5.57 (J=1.5 Hz)	1.99, 2.09, 2.17 (9H, Ac)
5a[a]	5.25 (J=5.4 Hz)	
5b[b]	not assigned	2.08 (3H, Ac), 2.14 (6H, Ac)
5c[b]	5.53 (J=5 Hz)	7.29 (15H, C_6H_5)
6a[a]	4.62 (J=8.2 Hz)	
6b[b]	not assigned	2.01 (6H, eq-Ac), 2.21 (3H, ax-Ac)

a) Measured in d_6-DMSO solution.
b) Measured in $CDCl_3$ solution.



1C — β anomer

C1 — α anomer

X: N($CH_2CH_2CH_2OCH_3$)($CONHCH_2CH_2Cl$)
Y: $COCH_3$

Fig. 1. Stable Conformers of L-Arabinopyranosyl Derivatives

β anomers[3] of L-arabinopyranosylureas. On the other hand, the presence of the anomeric proton signal of **3a** at δ 4.67 as a doublet (J=8.1 Hz) was compatible with its α anomer structure[3] (see Table II). These assignments were also supported by the chemical shifts of the acetoxyl protons of **1b** and **3b**. The axial acetoxyl protons on a pyranoid ring are known to appear at lower field (δ 2.15—2.2) than the equatorial ones (δ 2.0—2.1).[4] On the basis of the NMR data of **1b** and **3b** (Table II), **1b** was shown to have two axial and one equatorial acetoxyl groups, and likewise **3b** was concluded to have one axial and two equatorial ones. This shows that **1b** is the β anomer of L-arabinopyranoside having the stable 1C conformation (see Fig. 1). Thus, the structures of **1a** and **3a** were confirmed to be the β and α anomers of 3-(L-arabinopyranosyl)-1-(2-chloroethyl)-3-(3-methoxy-*n*-propyl)urea, respectively.

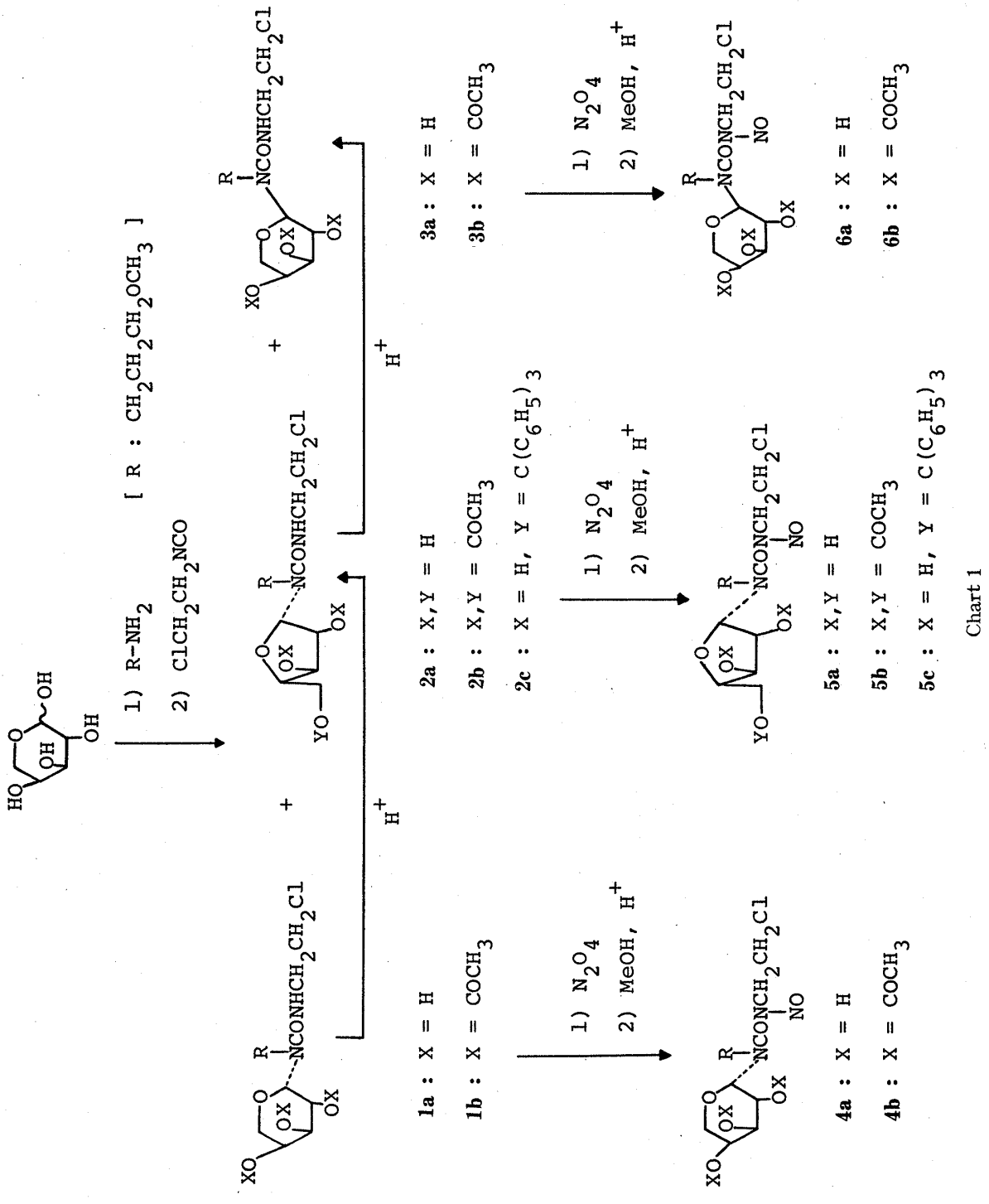


Chart 1

Finally, the structure of **2a** was determined to be the β anomer of 3-(L-arabinofuranosyl)-1-(2-chloroethyl)-3-(3-methoxy-*n*-propyl)urea from the signal of its anomeric proton at δ 5.37 (J=6.4 Hz) and those of **2b** and **2c** (see Table II), because the coupling constants ($J_{1,2}$) of the anomeric protons of the α and β anomers of methyl L-arabinofuranoside were reported to be 1.0 and 4.0 Hz, respectively.[5]

Isomerization of the ureas (**1a**, **2a**, and **3a**) into a thermodynamically stable isomer was examined in formic acid solution by monitoring their TLC. The spot of **1a** changed to those of **1a** and **2a** immediately after dissolving **1a** in formic acid. Similarly, partial isomerization of **2a** into **3a** was observed immediately.

In contrast, **3a** remained unchanged in formic acid (see Fig 2, C). Complete isomerization of both **1a** and **2a** into **3a** was observed after 7 min. These results indicate that **1a** isomerizes into **3a** *via* **2a**. Consequently, the isomerization of the mixture of these ureas into **3a** could readily be effected by formic acid treatment.

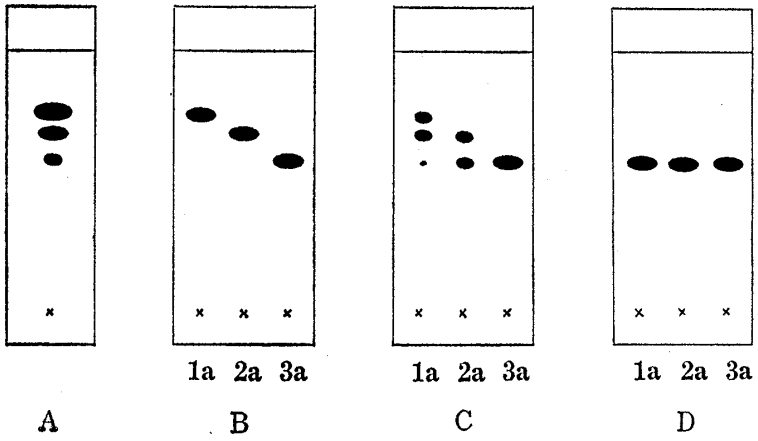


Fig. 2. Isomerization of the Urea Derivatives of L-Arabinose (**1a**, **2a** and **3a**) by Formic Acid (Thin-layer Chromatograms; Silica Gel, Solvent: AcOEt–EtOH–H_2O=8:2:1)

A: TLC of the mixture of the ureas before separation.
B: TLC of the ureas separated by column chromatography.
C: TLC of the ureas 1 min after dissolution in formic acid.
D: TLC of the ureas 7 min after dissolution in formic acid.

The nitrosation of these ureas by the use of dinitrogen tetroxide followed by treatment with methanol gave the corresponding nitrosoureas (**4a**, **5a**, and **6a**). The nitrosourea (**4a**) could not be isolated because of its instability. After acetylation of the crude product, however, its acetate (**4b**) could be isolated. This appears to support the β-L-arabinopyranosyl structure of **4a**, because the C-2 hydroxyl group of **4a** is close enough to the urea carbonyl group to cause facile decomposition by attack of the former on the latter.[2] The nitrosoureas (**5a** and **6a**) could be isolated and acetylated to give the corresponding acetates (**5b** and **6b**). As expected, **5a** gave the tritylated compound (**5c**). The mass and NMR spectral data of these nitrosoureas which are compatible with the assigned structures, are listed in Table I and Table II.

Further studies on a new class of nitrosoureas disubstituted on the N-3 position are in progress.

Experimental

Infared (IR) spectra were recorded with a Hitachi IR-215 spectrometer, NMR spectra with a JEOL PMX 60 spectrometer (using TMS as an internal standard), and mass spectra with a Hitachi RMU-6M spectrometer. The optical rotations were measured in a 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 TLC plate silica gel 60 F_{254} and 30% sulfuric acid was used as the spray reagent.

The Preparation and the Separation of L-Arabinosylureas (1a, 2a, and 3a)——A mixture of L-arabinose 6.0 g (0.04 mol), 3-methoxy-*n*-propylamine 3.9 g (0.044 mol), and 40 ml of methanol was stirred and heated at 65°C for 40 min. 2-Chloroethyl isocyanate 5.1 g (0.048 mol) was then added dropwise at 5°C and the whole was stirred for 1.5 h at room temperature and concentrated under reduced pressure. The residue was chromatographed on silica gel (solvent: AcOEt–EtOH–H_2O=30: 3: 1) to give the separated pure ureas (**1a**, **2a**, and **3a**) as colorless amorphous caramels. **1a**; yield 4.2 g. $[\alpha]_D^{17}$ −20.3° (c=1.8, methanol). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3350 (broad), 1630, 1550, 1070, 1000. NMR (DMSO-d_6) δ: 1.47—1.96 (m, 2H, $NCH_2C\underline{H}_2$), 3.24 (s, 3H, OCH_3), 5.35 (d, 1H, J=1.5 Hz, H-1), 6.51 (broad t, 1H, NH). **2a**; yield 2.5 g. $[\alpha]_D^{17}$ −28.7° (c=1.0, methanol). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3350 (broad), 1635, 1550, 1080, 1030. NMR (DMSO-d_6) δ: 1.64—2.06 (m, 2H, $NCH_2C\underline{H}_2$), 3.24 (s, 3H, OCH_3), 5.37 (d, 1H, J=6.4 Hz, H-1), 6.62 (broad t, 1H, NH). **3a**; yield 1.5 g. NMR (DMSO-d_6) δ: 1.62—1.86 (m, 2H, $NCH_2C\underline{H}_2$), 3.21 (s, 3H, OCH_3), 4.67 (d, 1H, J=8.1 Hz, H-1), 6.52 (broad t, 1H, NH).

Preparation of the Triacetates (1b, 2b, and 3b) of the Ureas (1a, 2a, and 3a)——A mixture of a urea (**1a**, **2a**, or **3a**) (1.0 g, 0.003 mol), acetic anhydride (5 ml), and pyridine (10 ml) was stirred at room temperature overnight. The reaction mixture was poured into water and extracted twice with 30 ml of ethyl acetate. The organic layer was washed with cold aqueous hydrochloric acid, water, and saturated NaCl solution, then dried over $MgSO_4$, and concentrated. The residue was purified by silica gel (solvent: AcOEt–Benzene–MeOH=10: 1: 1) to give the triacetate as an amorphous powder. **1b**; yield 0.8 g (57%). $[\alpha]_D^{20}$ −17.3° (c=1.1, methanol). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3350, 1750, 1645, 1530, 1230, 1075, 1060, 1025. NMR ($CDCl_3$) δ: 2.00 (s, 3H, eq-CH_3CO), 2.15 (s, 6H, ax-CH_3CO), 3.37 (s, 3H, OCH_3), 5.77 (d, 1H, J=2 Hz, H-1), 6.19 (broad t, 1H, NH). **2b**; yield 1.0 g (71%). $[\alpha]_D^{20}$ −13.4° (c=1.5, methanol). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3350, 1750, 1650, 1550, 1230, 1050. NMR ($CDCl_3$) δ: 2.09 (s, 9H, CH_3CO), 3.37 (s, 3H, OCH_3), 5.92 (d, 1H, J=5.2 Hz, H-1), 6.27 (broad t, 1H, NH). **3b**; yield 1.1 g (79%). $[\alpha]_D^{20}$ −35.8° (c=2.5, methanol). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3380, 1750, 1655, 1535, 1240, 1095, 1060, 1025. NMR ($CDCl_3$) δ: 2.00 (s, 6H, eq-CH_3CO), 2.16 (s, 3H, ax-CH_3CO), 3.36 (s, 3H, OCH_3), 6.24 (broad t, 1H, NH).

Reaction of the Urea (1a, 2a, and 3a) with Trityl Chloride in Pyridine——A mixture of a urea (650 mg), trityl chloride (1.7 g), and pyridine (7 ml) was stirred at room temperature for 3 d. Only **2a** could react with trityl chloride. The reaction mixture was poured into water and extracted twice with 20 ml of ethyl acetate. The organic layer was washed with dil. HCl, water, and NaCl solution, then dried over $MgSO_4$, and concentrated. The residual oil was purified on silica gel (solvent: AcOEt–benzene–MeOH=10:1: 1) to give the tritylated compound (**2c**) of **2a** in 65% yield as an amorphous powder. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3350, 1640, 1550, 1080, 1030, 1005, 705. NMR (DMSO-d_6) δ: 1.83—1.97 (m, 2H, $NCH_2C\underline{H}_2$), 3.25 (s, 3H, OCH_3), 5.51 (d, 1H, J=6.0 Hz, H-1), 6.60 (broad t, 1H, NH), 7.35 (s, 15H, phenyl protons).

Nitrosation of the L-Arabinosylureas (1a, 2a, and 3a)——The L-arabinosylurea (1.6 g, 0.005 mol) was dissolved in 20 ml of tetrahydrofuran and then anhydrous sodium acetate (1.6 g, 0.02 mol) was added. Dinitrogen tetroxide (2.0 g, 0.022 mol) was introduced into the mixture at −5°C for 10 min under vigorous stirring. After 10 min, 4 ml of methanol was added to the mixture. The whole was stirred at the same temperature for 10 min, then cold ethyl acetate (30 ml), anhydrous sodium acetate (0.8 g, 0.01 mol) and 5 ml of water at −5°C were added with stirring. The mixture was stirred vigorously for 10 min and its pH 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=5: 2: 1). The nitrosourea (**4a**) from **1a** could not be isolated in a pure form because of its instability. **5a** (from **2a**); yield 1.0 g (56%), a yellow caramel. $[\alpha]_D^{18}$ −34.2° (c=0.4, methanol). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3410, 1695, 1080, 1070, 1005. NMR (DMSO-d_6) δ: 1.63—1.95 (m, 2H, $NCH_2C\underline{H}_2$), 3.22 (s, 3H, OCH_3), 5.25 (d, 1H, J=5.4 Hz, H-1). **6a** (from **3a**); yield 1.3 g (73%). $[\alpha]_D^{20}$ +51.0° (c=1.1, methanol). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3380, 1700, 1095, 1075, 1030, 1015. NMR (DMSO-d_6) δ: 1.78—2.11 (m, 2H, $NCH_2C\underline{H}_2$), 3.21 (s, 3H, OCH_3), 4.62 (d, 1H, J=8.2 Hz, H-1).

Preparation of the Triacetates (4b, 5b, and 6b) of the Nitrosoureas (4a, 5a, and 6a)——The triacetates (**4b**, **5b**, and **6b**) were similarly obtained from the crude **4a**, **5a**, and **6a**, respectively, by the method described for the preparation of the triacetates (**1b**, **2b**, and **3b**) of the ureas. **4b**; yield 31% (from crude **4a**), a yellow amorphous powder. $[\alpha]_D^{18}$ +30.5° (c=0.9, methanol). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1755, 1700, 1500, 1250, 1070, 1025. NMR ($CDCl_3$) δ: 1.99 (s, 3H, eq-CH_3CO), 2.09 (s, 3H, ax-CH_3CO), 2.17 (s, 3H, ax-CH_3CO), 3.28 (s, 3H, OCH_3), 5.57 (d, 1H, J=1.5 Hz, H-1). **5b**; yield 62% (from **5a**), a yellow caramel. $[\alpha]_D^{18}$ −4.9° (c=1.0, methanol). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1750, 1705, 1505, 1230, 1060. NMR ($CDCl_3$) δ: 2.08 (s, 3H, CH_3CO), 2.14 (s, 6H, CH_3CO), 3.33 (s, 3H, OCH_3). **6b**; yield 71% (from **6a**), a yellow amorphous powder. $[\alpha]_D^{18}$ +48.4° (c=0.7, methanol). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1755, 1705, 1505, 1230, 1060. NMR ($CDCl_3$) δ: 2.01 (s, 6H, eq-CH_3CO), 2.21 (s, 3H, ax-CH_3CO), 3.29 (s, 3H, OCH_3).

Preparation of the Tritylated Compound (5c) of the Nitrosourea (5a)——The tritylated compound (**5c**) was similarly obtained from **5a** in 63% yield by the method described for the preparation of the tritylated compound (**2c**) of **2a**. $[\alpha]_D^{18}$ −37.7° (c=1.1, methanol). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3400, 1695, 1070, 1030, 1000. NMR ($CDCl_3$) δ: 1.92—2.15 (m, 2H, $NCH_2C\underline{H}_2$), 3.27 (s, 3H, OCH_3), 5.53 (d, 1H, J=5.0 Hz, H-1), 7.29 (s, 15H, phenyl protons).

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References and Notes

1) For part IV of this series, see K. Tsujihara, M. Ozeki, T. Morikawa, M. Kawamori, and Y. Arai, *J. Med. Chem.*, **25**, 441 (1982).
2) *a*) For part II of this series, see K. Tsujihara, M. Ozeki, T. Morikawa, N. Taga, M. Miyazaki, M. Kawamori, and Y. Arai, *Chem. Pharm. Bull.*, **29**, 3262 (1981); *b*) For part III of this series, see T. Morikawa, M. Ozeki, N. Umino, M. Kawamori, Y. Arai, and K. Tsujihara, *Chem. Pharm. Bull.*, **30**, 534 (1982).
3) Note that the nomenclature of anomers in the L-series is opposite to that in the D-series. Namely, β-L-arabinose and α-D-galactose have the same absolute configuration at C-1 and at the other asymmetric atoms.
4) S. Hirano, *Kagaku No Ryoiki*, **22**, 54 (1968).
5) W.G. Overend, "The Carbohydrates. Chemistry and Biochemistry," Vol. IA, 2nd ed., ed. by W. Pigman and D. Horton, Academic Press, Inc., New York, 1972, p. 309.