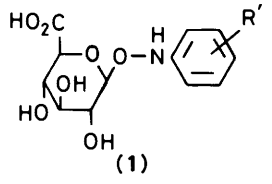
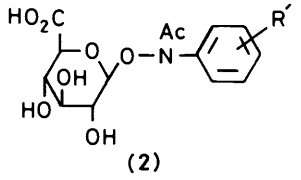
# Glycosides of *N*-Hydroxy-*N*-arylamine Derivatives. Part 2.[1] Convenient Synthetic Methods for *N*-Glycosides of *N*-Hydroxy-*N*-arylamines

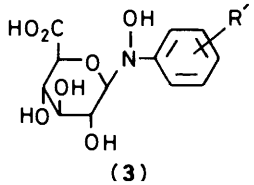
**Tadao Yoshioka, Hidetoshi Yamada, and Takayoshi Uematsu ***
*Department of Chemical Hygiene, Hokkaido Institute of Pharmaceutical Sciences, Otaru 047-02, Japan*

Two convenient methods for the synthesis of *N*-glucuronides of *N*-hydroxy-*N*-arylamines, (**8a**—**e**) and (**9a**), have been developed. *N*-Hydroxy-*N*-arylamines (**6a**—**e**) were condensed with triethylammonium D-glucopyranuronate (**4**) to give 1-deoxy-1-(*N*-hydroxy-*N*-arylamino)-β-D-glucopyranuronates (**8a**—**e**) (method A), and with D-glucofuranurono-6,3-lactone (**12**) to give 1-deoxy-1-(*N*-hydroxy-*N*-arylamino)-D-glucofuranurono-6,3-lactones (**14a**—**e**), which were also converted into the same pyranuronates as obtained by method A (method B). Pyridinium perchlorate in pyridine was a particularly effective acid catalyst in these condensation reactions, and also the *N*-glucoside (**11e**) was synthesized from D-glucose under the same conditions as those of method A. The mechanism of formation of compounds (**14a**—**e**) and (**8a**—**e**), and the ring transformation of compounds (**14a**—**e**) into compounds (**8a**—**e**), are briefly discussed.

In the preceding paper,[1] we have reported the synthesis of *O*-glucosides of *N*-hydroxy-*N*-arylamines and their hydroxamic acids by using an orthoester glycosylation method. In metabolic studies of carcinogenic nitro- and amino-aromatics, three types of glucuronides, *i.e.* (**1**), (**2**), and (**3**), have been demonstrated to be involved in carcinogenicity.[2,3] Although compound (**1**) has not been isolated as a metabolite, metabolic formation of compound (**1**) through enzymatic deacetylation of compound (**2**) *in vitro* has been suggested.[4,5] In metabolic studies of urinary bladder carcinogens, such as 2-naphthylamine and 4-aminobiphenyl, another type of glucuronide, compound (**3**), has been identified both *in vivo*[6,7] and *in vitro*.[8,9] These *N*-glucuronides are readily hydrolysed in acidic media or by β-glucuronidase to *N*-hydroxy-*N*-arylamines which in turn are converted into electrophilic nitrenium/carbonium ions; hence, *N*-glucuronides of *N*-hydroxy-*N*-arylamines, carriers of the ultimate carcinogen from the liver to the site of action, have been considered[3,10] as proximate carcinogens, especially for urinary bladder and colon cancers.


(**1**)


(**2**)


(**3**)

The first synthesis[9,11] of *N*-glucuronides of *N*-hydroxy-*N*-arylamines was achieved under neutral or slightly alkaline conditions, though in poor yield. Since it was reported that formation of *N*-glycosides of arylamines is favoured in acidic conditions[12] (optimum pH 3—4), an acid-catalysed condensation in an aprotic solvent was attempted. In this paper, convenient methods for the synthesis of the *N*-glycosides (**8a**—**e**), (**9a**), (**11e**), and *N*-hydroxy-*N*-arylaminoglucofuranurono-6,3-lactones (**14a**—**e**) are described.

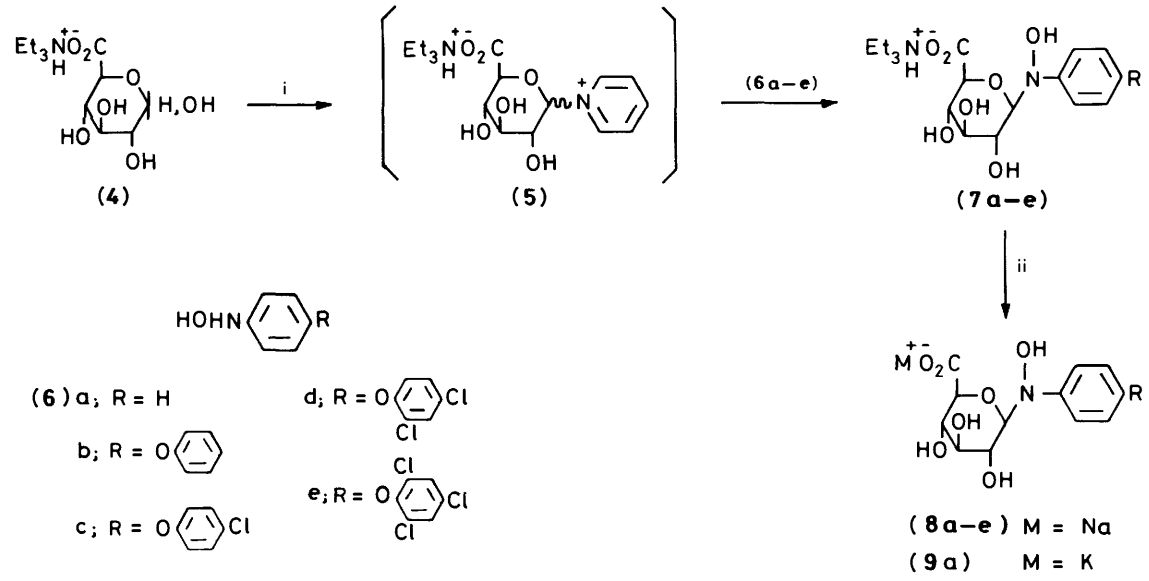
## Results and Discussion

*Reaction of the* N-*Hydroxy*-N-*arylamines* (**6a**—**e**) *with Triethylammonium* D-*Glucopyranuronate* (**4**). *Method A.*—Synthesis of the *N*-glucuronides of *N*-hydroxy-*N*-arylamines, (**8a**—**e**) and (**9a**) was carried out in pyridine in the presence of a catalytic amount of pyridinium perchlorate (0.14 mol equiv.) under nitrogen as shown in Scheme 1. Since *N*-glucuronides of *N*-hydroxy-*N*-arylamines were shown[6] to be labile in acidic media, being solvolysed to the parent compounds, the acid-catalysed condensation reaction in a pyridine-buffered system has been employed.

Triethylammonium D-glucopyranuronate (**4**) was used instead of its sodium salt because the latter showed poor solubility in pyridine. Pyridine was our choice of solvent because (i) it showed a moderate ability to dissolve both the starting materials, (ii) it showed no reactivity toward either starting material under the reaction conditions, and (iii) it was easily removable by evaporation under reduced pressure.

No reaction was observed in the absence of pyridinium perchlorate, a more effective catalyst than pyridinium sulphate or Dowex 50W-X8 (pyridinium form) resin. The $pK_a$ value of perchloric acid is lower than that of sulphuric acid by 5 $pK_a$ units, so perchloric acid seems to facilitate the formation of a carbonium ion or a pyridinium intermediate (**5**). Although this acid-catalysed reaction was thought to be reversible, the overall reaction favoured the forward direction probably due to the following reasons: (i) since pyridine is the most basic component in the reaction mixture ($pK_a$ values for pyridine and *N*-phenylhydroxylamine are 5.2 and 1.9, respectively), protonation of compounds (**7a**—**e**), a first step in the reverse reaction, seems to occur to a lesser extent. (ii) Pyridine forms a hydrate, which seems to function as a trapping reagent for active water.

As the primary products (**7a**—**e**) were difficult to crystallize, they were converted into isolable sodium (**8a**—**e**) and potassium (**9a**) salts on treatment with sodium carbonate or potassium carbonate, respectively, in good yields (Table 1). These compounds could be recrystallized from aqueous tetrahydrofuran (THF), or aqueous acetonitrile. It is important to note that these compounds are hydrolysed not only in acidic but also even in neutral solution. The structures of the compounds (**8a**—**e**) and (**9a**) were confirmed by their i.r. and $^1$H n.m.r. spectra. The i.r. spectra showed strong bands at 1 600—1 610 cm$^{-1}$ and 1 410—1 415 cm$^{-1}$, attributed to the carboxylate group. The $^1$H n.m.r. spectra of these compounds showed the *N*-hydroxy protons at $\delta_H$ 9.24—9.50 as broad singlets (exchangeable with




**Scheme 1.** *Reagents:* i, pyridinium perchlorate; ii, $Na_2CO_3$ or $K_2CO_3$

**Table 1.** Yields (%) of *N*-glucuronides of *N*-hydroxy-*N*-arylamines, (**8a**—**e**) and (**9a**)

| Compound | Yields[a] (%) Method A | Method B |
|---|---|---|
| (**8a**) | 90 | 96 |
| (**9a**) | 97 | 95 |
| (**8b**) | 78 | 72 |
| (**8c**) | 82 | 95 |
| (**8d**) | 77 | 78 |
| (**8e**) | 60 | 98 |

[a] Isolation yield from the reaction mixture (crude).

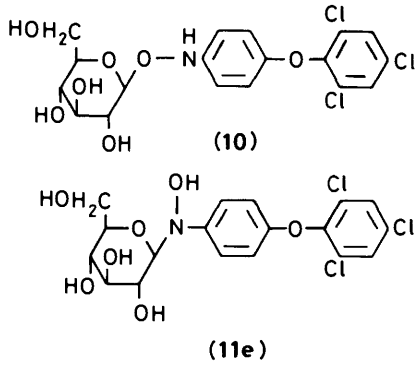



**Table 2.** Chemical shifts ($\delta_H$; 100 MHz) and coupling constants (*J*/Hz) of compounds (**14a**—**e**) in $(CD_3)_2SO$–$D_2O$ and of compound (**15a**) in $(CD_3)_2SO$

| Compound | 1-H | 2-H[b] | 3-H | 4-H | 5-H |
|---|---|---|---|---|---|
| (**14a**) | 5.38 (d, *J* 3.4)[a] | 4.45 (d, *J* 3.4) | 4.60—4.72 (2 H, m) | | 4.48 (d, *J* 3.9) |
| (**14b**) | 5.27 (d, *J* 3.2) | 4.44 (d, *J* 3.2) | 4.65—4.76 (2 H, m) | | 4.50 (d, *J* 3.8) |
| (**14c**) | 5.26 (d, *J* 3.2) | 4.42 (d, *J* 3.2) | 4.60—4.74 (2 H, m) | | 4.46 (d, *J* 3.8) |
| (**14d**) | 5.24 (d, *J* 3.4) | 4.42 (d, *J* 3.4) | 4.62—4.72 (2 H, m) | | 4.48 (d, *J* 3.9) |
| (**14e**) | 5.22 (d, *J* 3.4) | 4.38 (d, *J* 3.4) | 4.64—4.74 (2 H, m) | | 4.45 (d, *J* 4.2) |
| (**15a**) | 5.82 (d, *J* 2.4) | 5.22 (d, *J* 2.4) | 5.08 (d, *J* 4.2) | 4.88—5.03[c] (m) | 5.72 (d, *J* 4.9) |

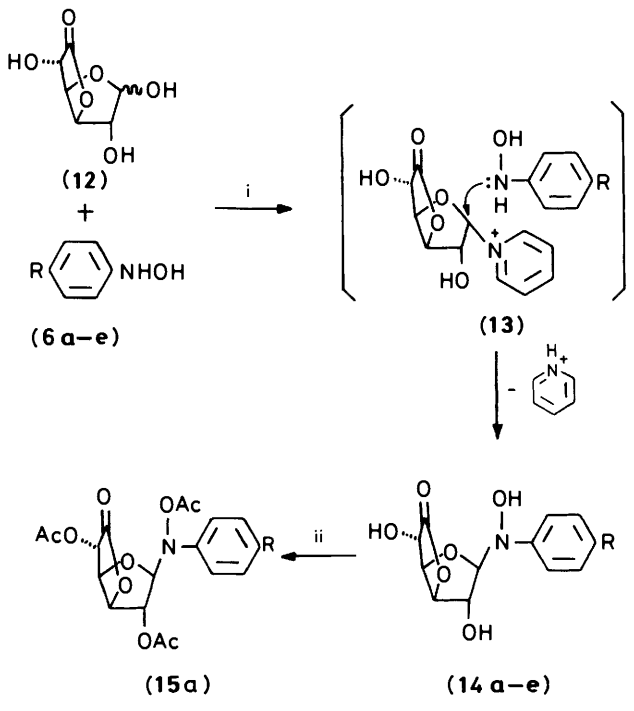
[a] Signal multiplicities: s, singlet; d, doublet; m, multiplet. [b] The signal collapses to a singlet by irradiation of the corresponding 1-H proton. [c] The signal collapses to a doublet by irradiation of the 5-H proton. 3-H and 4-H form the AB part of an ABX system (X = 5-H).

$D_2O$) and the anomeric protons at $\delta_H$ 4.59—4.68 as doublets with *J* 8.0—8.3 Hz, indicating[13] the β-configuration for all these compounds. Although the methine protons other than the anomeric proton resonated at higher field, they could not be further characterized. Reaction of the *N*-hydroxy-*N*-arylamine (**6e**) and D-glucose under the same conditions as described in method A gave the corresponding compound (**11e**) in good yield. The $^1H$ n.m.r. spectrum showed the *N*-hydroxy proton at $\delta_H$ 8.44 and the anomeric proton at $\delta_H$ 4.64 as a doublet with *J* 8.1 Hz. Enzymatic hydrolysis of compound (**11e**) with β-glucosidase (emulsin) gave D-glucose, whereas the glucosidic bond was not hydrolysed by α-glucosidase (yeast).

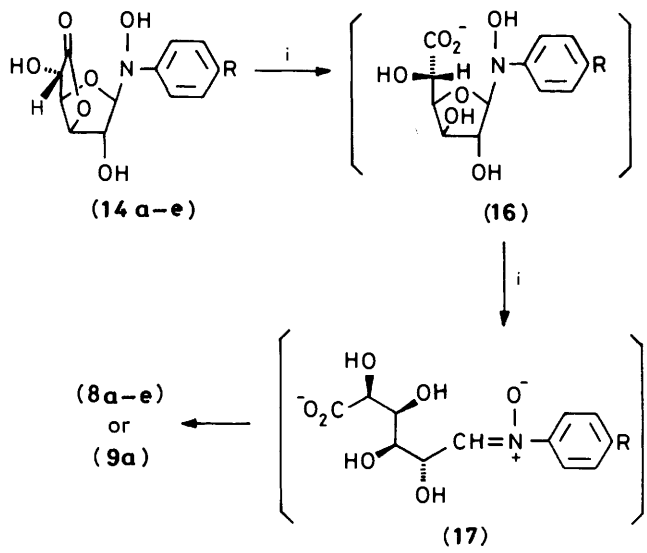
On silica gel t.l.c. with a variety of solvent systems, compound (**11e**) showed different $R_F$ values from those of the compound (**10**),[1] the N–O–C-1 isomer prepared *via* an orthoester method. From these facts, the structure of compound (**11e**) has been established as the β-D-glucopyranoside having an N–C-1 linkage in the molecule.

Under these conditions, no formation of nitrone derivatives[14] or Amadori rearrangement products[15] was observed. Furthermore, since compound (**11e**) was hydrolysed enzymatically by β-glucosidase and pyridine has been used as recrystallization solvent for D-glucose, it was thought that epimerization of the sugar moieties reported in this paper did not occur. From the $^1$H n.m.r. spectra of compounds (**8a—e**), (**9a**), and (**11e**), in which their anomeric protons resonated in a similar manner, the β-configuration for all the compounds obtained by method A was confirmed.

*Reaction of the* N-*Hydroxy*-N-*arylamines* (**6a-e**) *with* D-*Glucofuranurono*-6,3-*lactone* (**12**). *Method B.*—It was shown that condensation of D-glucofuranurono-6,3-lactone (**12**) with primary aromatic amines in polar media easily gave the corresponding 1-(*N*-arylamino)-1-deoxy-D-glucofuranurono-6,3-lactones.[16] The basicity of *N*-hydroxy-*N*-arylamines was lower than that of the corresponding arylamines (as described before), so the reaction time required for the formation of *N*-hydroxy-*N*-arylamine *N*-glycoside was so long[9,11,17] as to cause decomposition of the labile *N*-hydroxy-*N*-arylamine. Uematsu and co-workers[18] reported the synthesis of arylureido *N*-glucuronides from D-glucofuranurono-6,3-lactone, in which pyridine containing a catalytic amount of conc. sulphuric acid was employed for the first condensation step because of the poor nucleophilicity of arylureas. The reaction of the *N*-hydroxy-*N*-arylamines (**6a—e**) with D-glucofuranurono-6,3-lactone (**12**) under the same conditions as described by method A provided near quantitative yields of the corresponding 1-(*N*-hydroxy-*N*-arylamino)-1-deoxy-D-glucofuranurono-6,3-lactones (**14a—e**) (Scheme 2). *N*-Hydroxy-*N*-arylamines (**6a—e**) reacted with D-glucofuranurono-6,3-lactone (**12**) more readily than with the D-glucopyranuronate (**4**) under the same conditions. The i.r. spectra of compounds (**14a—e**) showed characteristic bands at 1 780—1 765 cm$^{-1}$ attributed to the γ-lactone group in the molecule, and their u.v. spectra resembled those of the corresponding *N*-hydroxy-*N*-arylamines (**6a—e**). The i.r. and $^1$H n.m.r. spectra of the per-acetylated compound (**15a**) also indicated the glucofuranuronolactone structure. In the i.r. spectrum, three bands at 1 810, 1 780, and 1 755 cm$^{-1}$ were attributed to *N*-acetoxy, γ-lactone, and *O*-acetyl functions, respectively. The $^1$H n.m.r. spectrum showed three methyl protons at $\delta_H$ 2.05, 2.12, and 2.17. An assignment of anomeric configuration to D-glucofuranuronolactone derivatives by means of $^1$H n.m.r. spectroscopy has been reported.[19,20] The anomeric proton was observed as a doublet with $J$ 3—5 Hz in the α-D-compounds, and as a singlet with $J \leq 0.5$ Hz in the β-D-compounds. These data suggested that the furanose ring of the D-glucofuranuronolactone derivatives favours the $^3T_2$ conformation,[21] and hence in the β-D-conformer the dihedral angle between the 1-H and 2-H bonds is *ca.* 90°. The $^1$H n.m.r. spectral data of compounds (**14a—e**) in $(CD_3)_2SO$–$D_2O$ are shown in Table 2. The protons at C-1—C-5 of the per-acetylated compound (**15a**) were assigned by using the homo-spin decoupling method and these data are also shown in Table 2. From the $J$ values of the anomeric protons, the α-configuration may be assigned for compounds (**14a—e**) and (**15a**). However, the optical rotation of compounds (**14a**), (**14b**), and (**14e**) was more laevorotatory than that of β-D-glucofuranurono-6,3-lactone, whereas that of 1-deoxy-1-(*N*-arylamino)-α-D-glucofuranurono-6,3-lactones[16] showed a positive large value, indicating the possibility of β-configuration for compounds (**14a—e**). From the values of the molecular rotation for compounds (**14a**), (**14b**), and (**14e**) and from the $^1$H n.m.r. spectra of compounds (**14a—e**), which showed a similar pattern, either of the two anomers was thought to be formed. As shown in Scheme 2, considering an intermediate (**13**) formed through nucleophilic approach of pyridine to the less hindered *exo* side of the D-glucofuranuronolactone (**12**), nucleophilic attack of compounds (**6a—e**) from the *endo* side would result in formation of compounds (**14a—e**) having the β-configuration. When an aglycone group becomes more bulky in β-D-glucofuranurono-6,3-lactone derivatives, steric repulsion between the aglycone group and the γ-lactone group is thought to increase for the $^3T_2$ conformer. Thus, compounds (**14a—e**) might have another type of conformation having a smaller degree of puckering in the THF ring. Since this assignment of the β-configuration was not unequivocal, the configuration of the compounds (**14a—e**) is under further investigation.




**Scheme 2.** *Reagents:* i, pyridinium perchlorate; ii, $Ac_2O$–pyridine. Compounds (**14a—e**) and (**15a**) may be *either* α- or β-anomers (see text). The β-anomer is shown




**Scheme 3.** *Reagent:* i, NaOH or KOH. The anomeric configuration of intermediate (**16**) is equivocal.

*Conversion of Compounds* (**14a**—**e**) *into* N-*Glucopyranuronates* (**8a**—**e**) *and* (**9a**).—Since it had been reported [22,23] that 1-deoxy-1-(*N*-arylamino)-D-glucofuranurono-6,3-lactones and -glucofuranuronamides were rapidly converted into the pyranose form in the presence of either acid or base, conversion of the furanosides (**14a**—**e**) into the corresponding pyranosides (**8a**—**e**) and (**9a**) was carried out by treatment with sodium hydroxide and potassium hydroxide, respectively. The reaction was complete within a few minutes. Yields of the products (**8a**—**e**) and (**9a**) prepared from D-glucofuranurono-6,3-lactone (**12**) (method B) without isolation of the furanosides (**8a**—**e**) are given in Table 1. These compounds (**8a**—**e**) and (**9a**) were identical by comparison of their spectral data with those of the products obtained by method A, and they did not show any m.p. depression on admixture. The mechanism of the ring-transformation reaction, similar to the mechanism reported by Uematsu and co-workers,[18] might be rationalized as shown in Scheme 3. After the addition of alkali the resultant mixture turned immediately to a deep orange solution and the colour then faded to yellow, indicating the formation of a nitrone intermediate (**17**). When aqueous sodium carbonate was added instead of aqueous sodium hydroxide, the reaction rate was decreased and on silica gel t.l.c. two main spots other than the starting material (**14a**) were detected. One had the same $R_F$ value as that of corresponding pyranoside (**8a**), and the other, with a higher $R_F$ value than that of the former, seemed to be the furanosiduronate intermediate (**16**) because this spot disappeared in the course of the reaction. From these facts, this ring-transformation reaction probably proceeded through hydrolysis of, first, the lactone ring and, second, the lactol ring and finally closure into the pyranoside form. It is thought that these methods could be generally applicable to the preparation of *N*-glucuronides and *N*-glucosides of *N*-hydroxy-*N*-arylamines. For the synthesis of the former, method B using commercially available D-glucofuranurono-6,3-lactone (**12**) is recommended.

## Experimental

General experimental directions are given in Part 1.[1]

*General Procedure for Preparation of* N-*Hydroxy*-N-*arylamines* (**6b**—**e**).—To a stirred solution of a nitro-aromatic compound $RC_6H_4NO_2$-*p* (0.1 mol) in dioxane (200 ml) and 2M-aqueous ammonium chloride (50 ml) at 50—60 °C was added, in portions, zinc dust (0.25 g-atom) during 10 min. After being stirred for an additional 15 min, the resultant mixture was filtrated through a Celite bed. The filtrate was evaporated to small volume under reduced pressure and the residue was taken up into benzene (40 ml). After the organic layer was dried ($Na_2SO_4$), hexane was added to give the corresponding *N*-hydroxy-*N*-arylamines (**6b**—**e**). *N*-Phenylhydroxylamine (**6a**) was synthesized by the reported procedure.[24] The physical data of compounds (**3b**—**e**) are given below.

N-*Hydroxy*-4-*phenoxyaniline* (**6b**), 45%, m.p. 71—74 °C (Found: C, 71.7; H, 5.5; N, 7.0. $C_{12}H_{11}NO_2$ requires C, 71.64; H, 5.47; N, 6.97%); $\lambda_{max.}$ (EtOH) 246 (ε 12 800), 276.5 (2 900), and 290.5 nm (2 000); $\nu_{max.}$ (KBr) 3 280, 3 160, 1 595, 1 510, 1 490, and 1 250 $cm^{-1}$; $\delta_H$ (100 MHz; $CDCl_3$) 7.31—6.90 (9 H, m) and 5.20 (2 H, br s, exchangeable with $D_2O$); *m*/*z* 201 ($M^+$), 199 ($M^+ - 2$), 185 ($M^+ - 16$, base peak), and 108 ($M^+ - 93$).

4-(4-*Chlorophenoxy*)-*N*-*hydroxyaniline* (**6c**), 24%, m.p. 96—97 °C (Found C, 61.3; H, 4.3; N, 5.8. $C_{12}H_{10}ClNO_2$ requires C, 61.65; H, 4.25; N, 5.94%); $\lambda_{max.}$ (EtOH) 246 (ε 14 000), 279 (3 000), and 288.5 nm (2 500); $\nu_{max.}$ (KBr) 3 280, 3 160, 1 595, 1 510, 1 490, and 1 260 $cm^{-1}$; $\delta_H$ (100 MHz; $CDCl_3$) 7.25 (2 H, d, *J* 9.0 Hz), 7.07—6.97 (4 H, m), 6.87 (2 H, d, *J* 9.0 Hz), and 5.31 (2 H, br s, exchangeable with $D_2O$); *m*/*z* 235 ($M^+$), 233 ($M^+ - 16$, base peak), and 108 ($M^+ - 125$).

4(2,4-*Dichlorophenoxy*)-N-*hydroxyaniline* (**6d**), 41%, m.p. 65—66 °C (Found: C, 53.5; H, 3.2; N, 4.9. $C_{12}H_9Cl_2NO_2$ requires C, 53.33; H, 3.33; N, 5.19%); $\lambda_{max.}$ (EtOH) 245 (ε 17 500), 284 (5 200), and 300 nm (2 800); $\nu_{max.}$ (KBr) 3 250, 3 140, 1 510, 1 480, and 1 260 $cm^{-1}$; $\delta_H$ (100 MHz; $CDCl_3$) 7.44 (1 H, d, *J* 2.4 Hz), 7.14 (1 H, dd, *J* 2.4 and 8.8 Hz), 7.07—6.95 (4 H, m), 6.80 (1 H, d, *J* 8.8 Hz), and 5.19 (2 H, br s, exchangeable with $D_2O$); *m*/*z* 269 ($M^+$), 267 ($M^+ - 2$), 253 ($M^+ - 16$, base peak), and 108 ($M^+ - 161$).

N-*Hydroxy*-4-(2,4,6-*trichlorophenoxy*)*aniline* (**6e**), 35%, m.p. 133—135 °C (Found: C, 47.4; H, 2.7; N, 4.5. $C_{12}H_8Cl_3NO_2$ requires C, 47.29; H, 2.63; N, 4.60%); $\lambda_{max.}$ (EtOH) 227.5 (ε 17 100), 236.5 (14 600), and 297 nm (3 100); $\nu_{max.}$ (KBr) 3 300, 3 100, 1 510, 1 450, and 1 265 $cm^{-1}$; $\delta_H$ (100 MHz; $CDCl_3$) 7.40 (2 H, s), 6.97 (2 H, d, *J* 9.0 Hz), 6.75 (2 H, d, *J* 9.0 Hz), and 4.52 (2 H, br s, exchangeable with $D_2O$); *m*/*z* 303 ($M^+$), 301 ($M^+ - 2$), 287 ($M^+ - 16$, base peak), and 108 ($M^+ - 195$).

*Preparation of Pyridinium Perchlorate Solution.*—Perchloric acid (60%; 2 ml, 18.5 mmol) was added to pyridine (30 ml) and the mixture was evaporated to dryness under reduced pressure. The residue was dissolved in pyridine (30 ml) and the solution was evaporated to dryness under reduced pressure. The residue was dissolved in pyridine (50 ml) and the solution was kept for use (final concentration 0.37 mM).

*Preparation of Triethylammonium* D-*Glucopyranuronate* (**4**).—A solution of sodium D-glucopyranuronate (20 mmol) in water (10 ml) was applied to a column of Dowex 50W-X8 ($NHEt_3^+$ form) (40 ml; height 24 cm) and eluted with water. An aliquot of the effluent containing the title compound (**4**) (2 mmol) was then evaporated under reduced pressure. The resultant residue was dissolved in pyridine and then evaporated under reduced pressure to remove traces of water, and afforded the title compound as a syrup.

*General Procedure for Preparation of* N-*Glucuronides of* N-*Hydroxy*-N-*arylamines*, (**8a**—**e**) *and* (**9a**).—*Method A.* To a solution of triethylammonium D-glucopyranuronate (**4**) (2 mmol) and an *N*-hydroxy-*N*-arylamine (**6a**—**e**) (2.4 mmol) in anhydrous pyridine (2 ml) was added the pyridinium perchlorate reagent (0.75 ml). After the solution had been stirred for 15 h at 37 °C under nitrogen, 1.5M-aqueous potassium hydrogen carbonate (0.2 ml) was added and the resultant precipitate was filtered off. Then, 1.1M-aqueous sodium carbonate (1.0 ml) was added to precipitate the title compounds as sodium salts, which were collected by filtration and washed with a small portion of pyridine, and then with ether. Compound (**9a**) was obtained by treatment with potassium carbonate instead of sodium carbonate in the above procedure.

*Method B.* To a solution containing D-glucofuranurono-6,3-lactone (**12**) (2 mmol) and an *N*-hydroxy-*N*-arylamine (**6a**—**e**) (2.4 mmol) in anhydrous pyridine (2 ml) was added the pyridinium perchlorate reagent (0.75 ml). After the solution had been stirred for 4 h at 37 °C under nitrogen, Dowex 1W-X8 ($HCO_3^-$ form) resin (180 mg, 0.58 mequiv.) was added and the mixture was stirred for an additional 30 min. After filtration to remove the resin, the stirred filtrate was treated with 1M-aqueous sodium hydroxide (2.1 ml) in an ice-bath to precipitate (immediately) the title compounds as sodium salts, which were collected by filtration and washed with a small portion of pyridine and then with ether. Compound (**9a**) was obtained by treatment with potassium hydroxide instead of sodium hydroxide in the above procedure. Thus prepared were *sodium* 1-*deoxy*-1-(N-*hydroxyanilino*)-β-D-*glucopyranuronate* (**8a**), m.p. 115 °C (decomp.) (from aqueous $CH_3CN$) (Found: C, 44.4; H,

5.1; N, 3.8. $C_{12}H_{14}NNaO_7 \cdot H_2O$ requires C, 44.31; H, 4.96; N, 4.31%); $\nu_{max.}$ (KBr) 3 350, 1 600, 1 490, 1 415, and 1 080 $cm^{-1}$; $\delta_H$(100 MHz; $(CD_3)_2SO$] 9.40 (1 H, br s, exchangeable with $D_2O$), 7.30—6.68 (5 H, m), 6.45 (1 H, br s, exchangeable with $D_2O$), 4.88 (2H, br s, exchangeable with $D_2O$), 4.68 (1 H, d, *J* 8.0 Hz), and 3.80—3.06 (m, overlapped with water peak).

*Potassium* 1-*deoxy*-1-(N-*hydroxyanilino*)-β-D-*glucopyranuronate* (**9a**), m.p. 158 °C (decomp.) (from aqueous EtOH) (Found: C, 42.6; H, 4.9; N, 4.0. $C_{12}H_{14}KNO_7 \cdot H_2O$ requires C, 42.22; H, 4.72; N, 4.10%); $\nu_{max.}$ (KBr) 3 350, 1 600, 1 495, 1 410, and 1 080 $cm^{-1}$; $\delta_H$ [100 MHz; $(CD_3)_2SO$] 9.24 (1 H, br s, exchangeable with $D_2O$), 7.28—6.72 (5 H, m), 6.41 (1 H, br s, exchangeable with $D_2O$), 4.88 (2 H, br s, exchangeable with $D_2O$), 4.68 (1 H, d, *J* 8.0 Hz), and 3.76—3.04 (m, overlapped with water peak).

*Sodium* 1-*deoxy*-1-(N-*hydroxy*-4-*phenoxyanilino*)-β-D-*glucopyranuronate* (**8b**), m.p. 101 °C (decomp.) (from aqueous THF) (Found: C, 50.9; H, 5.5; N, 2.9. $C_{18}H_{18}NNaO_8 \cdot \frac{3}{2}H_2O$ requires C, 50.70; H, 4.97; N, 3.29%); $\nu_{max.}$ (KBr) 3 350, 1 610, 1 490, 1 415, 1 240, and 1 075 $cm^{-1}$; $\delta_H$ [100 MHz; $(CD_3)_2SO$] 9.50 (1 H, br s, exchangeable with $D_2O$), 7.42—6.88 (9 H, m), 6.48 (1 H, br s, exchangeable with $D_2O$), 4.90 (2 H, br s, exchangeable with $D_2O$), 4.66 (1 H, d, *J* 8.2 Hz), and 3.58—3.04 (m, overlapped with water peak).

*Sodium* 1-*deoxy*-1-[4-(4-*chlorophenoxy*)-N-*hydroxyanilino*]-β-D-*glucopyranuronate* (**8c**), m.p. 105 °C (decomp.) (from aqueous THF) (Found: C, 48.8; H, 4.4; N, 2.8. $C_{18}H_{17}ClNNaO_8 \cdot \frac{1}{2}H_2O$ requires C, 48.82; H, 4.10; N, 3.16%); $\nu_{max.}$ (KBr) 3 400, 1 610, 1 510, 1 440, 1 250, and 1 080 $cm^{-1}$; $\delta_H$ [100 MHz; $(CD_3)_2SO$] 9.46 (1 H, br s, exchangeable with $D_2O$), 7.38 (2 H, d, *J* 8.8 Hz), 7.12 (2 H, d, *J* 8.8 Hz), 6.92 (2 H, d, *J* 8.8 Hz), 6.90 (2 H, d, *J* 8.8 Hz), 6.36 (1 H, br s, exchangeable with $D_2O$), 4.96 (2 H, br s, exchangeable with $D_2O$), 4.67 (1 H, d, *J* 8.3 Hz), and 3.04—3.60 (m, overlapped with water peak).

*Sodium* 1-*deoxy*-1-[4-(2,4-*dichlorophenoxy*)-N-*hydroxyanilino*]-β-D-*glucopyranuronate* (**8d**), m.p. 122 °C (decomp.) (from aqueous THF) (Found: C, 46.1; H, 4.0; N, 2.9. $C_{18}H_{16}Cl_2NNaO_8$ requires C, 46.17; H, 3.45; N, 2.99%); $\nu_{max.}$ (KBr) 3 400, 1 610, 1 510, 1 440, 1 260, and 1 080 $cm^{-1}$; $\delta_H$ [100 MHz; $(CD_3)_2SO$] 9.40 (1 H, br s, exchangeable with $D_2O$), 7.69 (1 H, d, *J* 2.5 Hz), 7.35 (1 H, dd, *J* 2.5 and 9.0 Hz), 7.12 (1 H, d, *J* 9.0 Hz), 6.86—6.98 (4 H, m), 6.40 (1 H, br s, exchangeable with $D_2O$), 4.88 (2 H, br s, exchangeable with $D_2O$), 4.68 (1 H, d, *J* 8.0 Hz), and 3.02—3.72 (m, overlapped with water peak).

*Sodium* 1-*deoxy*-1-[N-*hydroxy*-4-(2,4,6-*trichlorophenoxy*)*anilino*-β-D-*glucopyranuronate* (**8e**), m.p. 138 °C (decomp.) (from aqueous THF) (Found: C, 42.9; H, 3.2; N, 2.75. $C_{18}H_{15}Cl_3NNaO_8$ requires C, 43.01; H, 3.01; N, 2.79%); $\nu_{max.}$ (KBr) 3 400, 1 610, 1 505, 1 445, 1 260, and 1 080 $cm^{-1}$; $\delta_H$ [100 MHz; $(CD_3)_2SO$] 9.44 (1 H, br s, exchangeable with $D_2O$), 7.81 (2 H, s), 7.03 (2 H, d, *J* 9.0 Hz), 6.68 (2 H, d, *J* 9.0 Hz), 6.40 (1 H, br s, exchangeable with $D_2O$), 4.96 (2 H, br s, exchangeable with $D_2O$), 4.64 (1 H, d, *J* 8.3 Hz), and 3.04—3.52 (m, overlapped with water peak).

1-*Deoxy*-1-[N-*hydroxy*-4-(2,4,6-*trichlorophenoxy*)*anilino*]-β-D-*glucopyranose* [N-*Hydroxy*-N-(2′,4′,6′-*trichlorobiphenyl*-4-*yl*)-β-D-*glucopyranosylamine*] (**11e**).—To a solution of D-glucose (2 mmol) and compound (**6e**) (2.4 mmol) in anhydrous pyridine (1 ml) was added the pyridinium perchlorate reagent in pyridine solution (0.75 ml, 0.27 mmol). After the solution had been stirred for 6 h at 37 °C under nitrogen, Dowex 1W-X8 ($HCO_3^-$ form) (180 mg, 0.58 mequiv.) was added and the mixture was stirred for an additional 15 min. After filtration to remove the resin, the filtrate was evaporated under reduced pressure and trituration of the residue with ethanol gave an insoluble solid which, on recrystallization from aqueous $CH_3CN$, gave the *title compound* (**11e**) (863 mg, 93%), m.p. 127 °C (decomp.) (Found: C, 45.5; H, 3.8; N, 3.0. $C_{18}H_{18}Cl_3NO_7 \cdot \frac{1}{2}H_2O$ requires C, 45.45; H, 4.03; N, 2.94%); $\nu_{max.}$ (KBr) 3 350, 2 900, 1 550, 1 500, 1 400, and 1 260 $cm^{-1}$; $\lambda_{max.}$ (60% aqueous $CH_3CN$) 276sh (ε 2 400) and 287 nm (2 300); $\delta_H$ [100 MHz; $(CD_3)_2SO$] 8.44 (1 H, br s, exchangeable with $D_2O$), 7.83 (2 H, s), 7.00 (2 H, d, *J* 9.0 Hz), 6.69 (2 H, d, *J* 9.0 Hz), 4.64 (1 H, d, *J* 8.1 Hz), 3.90—3.16 (m, overlapped with water peak).

*Enzymatic Hydrolysis of the* N-*Glucoside* (**11e**).—A solution of the N-glucoside (**11e**) (0.1 ml, final concentration 1.01 mM) was added to 0.1M-aqueous sodium acetate buffer (pH 5.5) (0.7 ml). After the addition of β-glucosidase (0.2 ml) (emulsin, from Sigma, 1.8 units), the solution was incubated at 37 °C for 3 h. During the incubation, the amount of liberated glucose was determined by the Somogy–Nelson method [25] after 15, 30, 60, 120, and 180 min. At the times indicated, charcoal (10 mg) to remove both the unchanged glucoside and liberated aglycone and Celite (40 mg) were added to the incubation mixture. After centrifugation of the suspension (2 500 r.p.m. for 3 min), an aliquot (0.5 ml) of the supernatant was taken for assay. The amount of glucose liberated by enzymatic hydrolysis of compound (**11e**) was higher than that by non-enzymatic hydrolysis (*i.e.* without β-glucosidase). The rates of hydrolysis with and without β-glucosidase were 0.10 μmol $h^{-1}$ (1.8 units), and 0.004 μmol $h^{-1}$, respectively.

*General Procedure for Preparation of* 1-*Deoxy*-1-(N-*hydroxy*-N-*arylamino*)-D-*glucofuranurono*-6,3-*lactones* (**14a**—**e**).—Following a procedure similar to that of method B for the preparation of compounds (**8**) and (**9a**) (above), the title compounds (**14a**—**e**) were isolated as follows. After filtration to remove the resin, the filtrate was evaporated under reduced pressure; trituration of the residue with ether gave an insoluble solid which, on recrystallization, gave the title compounds (**14a**), (**14b**), and (**14e**). Compounds (**14c**) and (**14d**) could not be made to recrystallize. The following lactones were prepared.

1-Deoxy-1-(N-hydroxyanilino)-D-glucofuranurono-6,3-lactone (**14a**), 100%, m.p. 108 °C (decomp.) (from ethyl acetate) (lit.,[17] 106 °C) (Found: C, 53.5; H, 5.1; N, 4.7. Calc. for $C_{12}H_{13}NO_6$: C, 53.93; H, 4.90; N, 5.24%); $[\alpha]_D^{17}$ −33.3° [*c* 1.4 in $CH_3CN$–water (3:1)]; $\lambda_{max.}$ ($CH_3CN$) 237 (ε 7 500) and 274 nm (800); $\nu_{max.}$ (KBr) 3 400, 1 770, 1 600, 1 495, and 1 135 $cm^{-1}$; $\delta_H$ [100 MHz; $(CD_3)_2SO$] 8.86 (1 H, s, exchangeable with $D_2O$), 7.25—6.90 (5 H, m), 5.38 (1 H, d, *J* 3.4 Hz), 4.60—4.80 (2 H, m), 4.44—4.59 (2 H, m), and 3.20—4.40 (2 H, br s, exchangeable with $D_2O$).

1-*Deoxy*-1-[N-*hydroxy*-4-*phenoxyanilino*]-D-*glucofuranurono*-6,3-*lactone* (**14b**), 100%, m.p. 74 °C (decomp.) (from $CH_3CN$) (Found: C, 60.0; H, 5.0; N, 6.8. $C_{18}H_{17}NO_7 \cdot CH_3CN$ requires C, 60.00; H, 5.04; N, 7.00%); $\lambda_{max.}$ ($CH_3CN$) 244 (ε 14 000) and 275sh nm (1 900); $\nu_{max.}$ (KBr) 3 360, 2 250 (solvate C≡N), 1 770, 1 585, 1 505, 1 490, and 1 240 $cm^{-1}$; $[\alpha]_D^{17}$ −42.1° [*c* 0.98 in $CH_3CN$–water (3:1)]; $\delta_H$ [100 MHz; $(CD_3)_2SO$] 8.86 (1 H, s, exchangeable with $D_2O$), 7.43—6.89 (9 H, m), 5.84 (1 H, d, exchangeable with $D_2O$), 5.66 (1 H, d, exchangeable with $D_2O$), 5.27 (1 H, d, *J* 3.2 Hz), 4.76—4.32 (4 H, m), and 2.06 (3 H, s, $CH_3CN$).

1-[4-(4-Chlorophenoxy)-*N*-hydroxyanilino]-1-deoxy-D-glucofuranurono-6,3-lactone (**14c**), 85%; $\nu_{max.}$ (KBr) 3 400, 1 765, 1 590, 1 485, and 1 245 $cm^{-1}$; $\delta_H$ [100 MHz; $(CD_3)_2SO$] 8.88 (1 H, s, exchangeable with $D_2O$), 7.39 (2 H, d, *J* 8.8 Hz), 7.20 (2 H, d, *J* 8.8 Hz), 6.96 (4 H, d, *J* 8.8 Hz), 5.50 (2 H, br s, exchangeable with $D_2O$), 5.25 (1 H, d, *J* 3.2 Hz), 4.60—4.76 (2 H, m), and 4.32—4.60 (2 H, m).

1-Deoxy-1-[4-(2,4-dichlorophenoxy)-*N*-hydrocyanilino]-D-glucofuranurono-6,3-lactone (**14d**), 82%; $\nu_{max.}$ (KBr) 3 350, 1 780, 1 500, 1 470, and 1 255 $cm^{-1}$; $\delta_H$ [100 MHz; $(CD_3)_2SO$] 8.84 (1 H, s, exchangeable with $D_2O$), 7.64 (1 H, d, *J* 2.4 Hz), 7.40 (1 H, dd, *J* 2.4 and 8.5 Hz), 7.16 (2 H, d, *J* 9.0 Hz), 6.88—7.04

(3 H, m), 5.60 (2 H, br s, exchangeable with $D_2O$), 5.27 (1 H, d, *J* 3.3 Hz), 4.58—4.78 (2 H, m), and 4.58—4.20 (2 H, m).

1-*Deoxy*-1-[N-*hydroxy*-4-(2,4,6-*trichlorophenoxy*)*anilino*]-D-*glucofuranurono*-6,3-*lactone* (**14e**), 100%, m.p. 92 °C (decomp.) (from $CH_3CN$) (Found: C, 47.4; H, 3.3; N, 5.2. $C_{18}H_{14}Cl_3NO_7$·$CH_3CN$ requires C, 47.69; H, 3.40; N, 5.56%); $[\alpha]_D^{17}$ −42.5° [*c* 1.21 in $CH_3CN$–water (3:1)]; $\lambda_{max.}$ ($CH_3CN$) 236sh (ε 15 700), 277 (2 600), and 286 nm (2 400); $\nu_{max.}$ (KBr) 3 400, 2 250 (solvate C≡N), 1 770, 1 505, 1 440, and 1 260 $cm^{-1}$; $\delta_H$ [100 MHz; $(CD_3)_2SO$] 8.78 (1 H, s, exchangeable with $D_2O$), 7.80 (2 H, s), 7.10 (2 H, d, *J* 9.0 Hz), 6.73 (2 H, d, *J* 9.0 Hz), 5.92 (1 H, br s, exchangeable with $D_2O$), 5.60 (1 H, br s, exchangeable with $D_2O$), 5.20 (1 H, d, *J* 3.5 Hz), 4.78—4.58 (2 H, m), and 4.58—4.20 (2 H, m), and 2.07 (3H, s, MeCN).

1-(N-*Acetoxyanilino*)-2,5-*di*-O-*acetyl*-1-*deoxy*-D-*glucofuranurono*-6,3-*lactone* (**15a**).—To an ice-cooled solution of compound (**14a**) (140 mg, 0.52 mmol) in pyridine (2 ml) was added acetic anhydride (0.5 ml, 8.8 mmol). After the mixture had been stirring for 1.5 h, methanol (0.32 ml, 7.9 mmol) was added and the mixture was stirred for an additional 15 min. The reaction mixture was evaporated under reduced pressure and the residue was taken up into ethyl acetate (30 ml). The organic solution was washed successively with 0.1M-aqueous HCl, saturated aqueous $NaHCO_3$, and water. After being dried ($Na_2SO_4$), the organic solution was evaporated under reduced pressure to give the *title compound* (**15a**) (176 mg, 86%), m.p. 139—139.5 °C (from ethyl acetate–hexane) (Found: C, 54.9; H, 4.95; N, 3.4. $C_{18}H_{19}NO_9$ requires C, 54.96; H, 4.87; N, 3.56%); $[\alpha]_D^{11}$ + 17.4° (*c* 0.94 in $CHCl_3$); $\lambda_{max.}$ (EtOH) 229 (ε 5 260) and 270sh nm (450); $\nu_{max.}$ (KBr) 2 950, 1 810, 1 780, 1 755, 1 590, 1 490, 1 370, and 1 210 $cm^{-1}$.

## References


1 Part 1, T. Yoshioka and T. Uematsu, preceding paper.
2 D. Malejka-Giganti, in 'Chemistry and Biology of Hydroxamic Acids,' ed. H. Kehl, Karger, Kirsville, Missouri, 1982, p. 149.
3 C. B. Kasper and D. Henton, in 'Enzymatic Basis of Detoxication,' ed. W. B. Jakoby, Academic Press, New York, 1980, vol. 2, p. 3.
4 C. C. Irving and L. T. Russel, *Biochemistry*, 1970, **9**, 2471.
5 R. A. Cardona and C. M. King, *Biochem. Pharmacol.*, 1976, **25**, 1051.
6 J. L. Radomski, A. A. Rey, and E. Brill, *Cancer Res.*, 1973, **33**, 1284.
7 J. L. Radomski, W. L. Hearn, T. Radomski, H. Moreno, and W. E. Scott, *Cancer Res.*, 1977, **37**, 1757.
8 F. F. Kadlubar, J. A. Miller, and E. C. Miller, *Cancer Res.*, 1977, **37**, 805.
9 J. M. Poupko, W. L. Hearn, and J. L. Radomski, *Toxicol. Appl. Pharmacol.*, 1979, **50**, 479.
10 F. F. Kadlubar, L. E. Unruh, T. J. Flammang, D. Sparks, R. K. Mitchum, and G. J. Mulder, *Chem.-Biol. Interact.*, 1981, **33**, 129.
11 H. R. Moreno and J. L. Radomski, *Cancer Lett.*, 1978, **4**, 85.
12 J. W. Bridges and R. T. Williams, *Biochem. J.*, 1962, **83**, 27.
13 M. Karplus, *J. Am. Chem. Soc.*, 1963, **85**, 2870.
14 H. Shindo and B. Umezawa, *Chem. Pharm. Bull.*, 1962, **10**, 492.
15 J. E. Hodge, *Adv. Carbohydr. Chem.*, 1955, **10**, 169.
16 K. Dax and H. Weidmann, *Adv. Carbohydr. Chem. Biochem.*, 1976, **33**, 190.
17 E. Boyland and R. Nery, *J. Chem. Soc. C*, 1963, 3141.
18 M. Akagi, I. Aoki, T. Uematsu, and T. Iyanagi, *Chem. Pharm. Bull.*, 1966, **14**, 18.
19 H. Weidmann and K. Dax, *Monatsh. Chem.*, 1971, **102**, 877.
20 D. Keglevic, D. Ljevakovic, and S. Tomic-Kulenovic, *Carbohydr. Res.*, 1981, **92**, 51.
21 R. J. Abraham and K. A. McLauchan, *Chem. Ind. (London)*, 1962, 213.
22 S. Takitani, *Chem. Pharm. Bull.*, 1961, **9**, 222.
23 T. Kishikawa, *Chem. Pharm. Bull.*, 1969, **17**, 2494.
24 O. H. Wheeler and P. H. Gore, *J. Am. Chem. Soc.*, 1956, **78**, 3363.
25 J. E. Hodge and B. T. Hofreiter, in 'Methods in Carbohydrate Chemistry,' eds. R. L. Whistler and M. L. Wolform, Academic Press, New York and London, 1962, vol. 1, p. 380.


*Received* 25*th June* 1984; *Paper* 4/1075