Glycosides of *N*-Hydroxy-*N*-arylamine Derivatives. Part 1. Synthesis and Mutagenicity of *O*-Glucosides of *N*-Hydroxy-*N*-arylamines and their Acetohydroxamic Acids

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N-Acetyl-*N*-arylamino β-D-glucopyranosides (**7a—d**) were synthesized by the orthoester glycosylation method *via* *N*-arylamino β-D-glucopyranosides (**6a—d**), and the *N*-acetyl amides, having an N–O–C-1 linkage in their molecules, were characterized by chemical, enzymatic, and spectral analyses. In the mutation assay using *Salmonella typhimurium* TA100 strain with or without various intracellular fractions of guinea pig liver, these glucosides (**7a—d**) were non-mutagenic *per se*, but showed mutagenic activity in the presence of the post-mitochondrial supernatant (S9) or the microsomal fraction (Ms), except for the glucoside (**7a**). Of the glucosides (**7b—d**), the compounds having fewer chlorine atoms were more effective in inducing mutations than were those having multiple chlorines. No mutagenic activity was observed in the presence of the soluble supernatant fraction (S10.5). The mutagenicity of the glucosides (**7b—d**) seemed to be due to the corresponding *N*-deacetylated compounds (**6b—d**) formed through hydrolysis by a microsomal deacetylase(s). The pathway of the metabolic activation of the glucosides (**7b—d**) is discussed.

In carcinogenicity of nitro- and amino-aromatics, *N*-hydroxy-*N*-arylamines formed metabolically through reductive and oxidative reactions, respectively, and their acetylated compounds, namely *N*-hydroxy-*N*-arylacetamides, are insufficiently electrophilic to be capable of interacting covalently with nucleophilic sites of cellular macromolecules and hence are said to be proximate carcinogens. The ultimate carcinogens are postulated[1,2] to be *N*–*O*-esterified products, such as glycosides, sulphates, and acetates. Although *O*-glucuronides of several *N*-hydroxy-*N*-arylacetamides have been obtained biosynthetically,[3–8] chemical synthesis of these compounds has not been reported and their chemical and biological characteristics have not been fully investigated. Since glucose conjugation has been known[9,10] to occur to some extent in mammals as well as insects and plants, it seems to be important to determine whether or not *O*-glucosides of *N*-hydroxy-*N*-arylacetamides, in addition to the corresponding *O*-glucuronides, participate in carcinogenicity. In this report, we report the synthesis, by the orthoester glycosylation method,[11] and the mutagenicity of the *O*-glucosides of acetohydroxamic acid derivatives of chlorinated 4-nitrobiphenyl ethers, widespread herbicides[12] used all over the world, one of which, 4-(2,4-dichlorophenoxy)nitrobenzene, has been reported to be carcinogenic.[13]

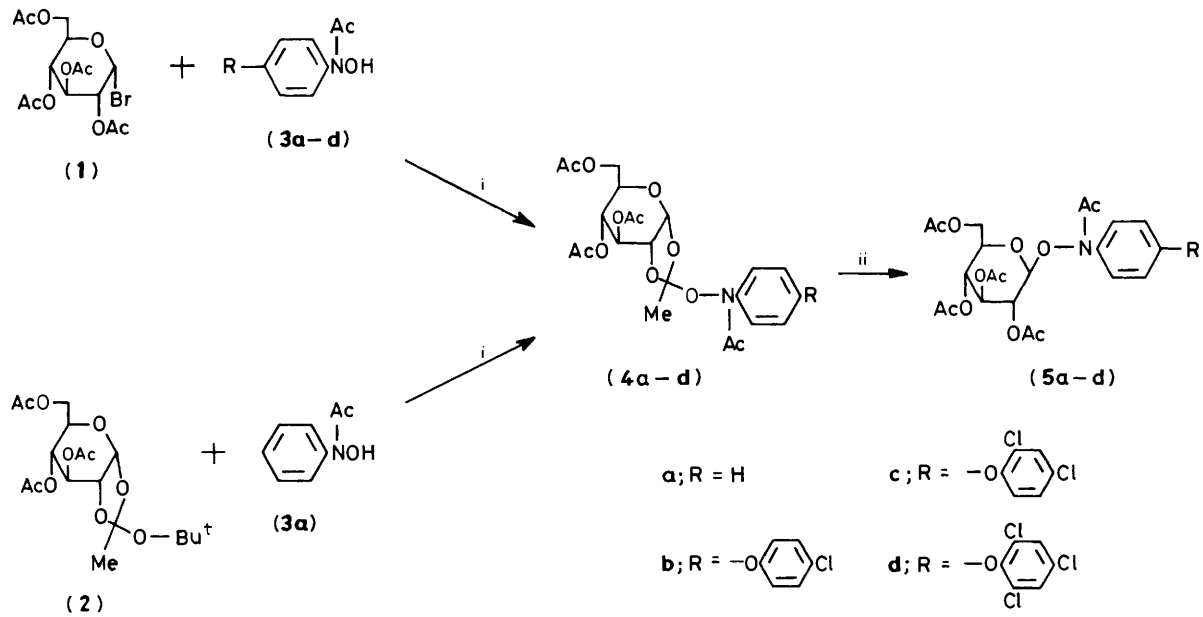
Results

Synthesis of 2,3,4,6-*Tetra*-O-*acetyl*-β-D-*glucopyranosides of* N-*Hydroxy*-N-*arylacetamides*, (**5a—d**).—Orthoesters (**4a—d**), key intermediates for the synthesis of the compounds (**5a—d**), were obtained in good yields from the corresponding *N*-hydroxy-*N*-arylacetamides (**3a—d**) and acetobromoglucose (**1**) as shown in Scheme 1. Rearrangement of the orthoesters (**4a—d**) to the corresponding tetra-*O*-acetyl-β-D-glucosides (**5a—d**) was achieved by heating under reflux in ethylene dichloride in the presence of a catalytic amount of 2,6-lutidinium perchlorate,[11] the most effective catalyst of all those examined. On the other hand, the glucoside (**5a**) could be synthesized by an alternative approach[11] using the t-butyl orthoester (**2**). In the reaction course, the orthoester (**4a**) was formed *in situ* by azeotropic removal of t-butyl alcohol and was then converted into the glucoside (**5a**) without isolation of the orthoester (**4a**). However, the yield of the glucoside (**5a**) based on the amount of compound (**3a**) consumed was less than that obtained by the method involving isolation of the orthoester (**4a**). Attempts to synthesize the glucosides (**5a—d**) by the Koenigs–Knorr method[14] resulted in poor yields.

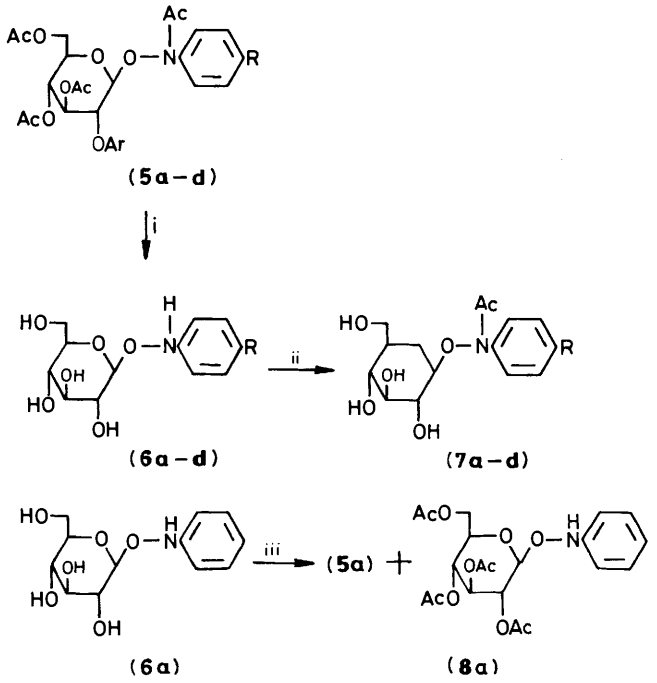
Synthesis of O-*Glucosides of* N-*Hydroxy*-N-*arylamines* (**6a—d**).—In methanol containing aqueous sodium hydroxide compound (**5a**) gave azoxybenzene, indicating that hydrolysis of both *O*- and *N*-acetyl groups followed by cleavage of the glucosidic bond was occurring. However, in anhydrous methanol containing barium methoxide (0.1 equiv.), or saturated with ammonia, compound (**5a**) gave the fully deacetylated compound (**6a**) in good yield though cleavage of the glucosidic bond occurred to a small extent. The compounds (**6b—d**), obtained from the corresponding compounds (**5b—d**) in this manner, were so labile as to decompose gradually even in neutral aqueous solution.

Selective N-*Acetylation of Compounds* (**6a—d**).—As shown in Scheme 2, compound (**6a**) was again per-acetylated to give products (**5a**) and (**8a**). Compound (**8a**), which is labile, could be also converted into compound (**5a**) by further acetylation. Synthesis of compound (**7a**) was achieved by selective *N*-acetylation of amine (**6a**) using a slight excess of acetyl chloride in the presence of sodium hydrogen carbonate and powdered molecular sieves (type 4Å) as an acid scavenger and a dehydrating reagent, respectively. In the case of compounds (**6b—d**), the acetylation was performed with the addition of triethylamine to the above reaction mixture as an additional acid scavenger. In the absence of triethylamine, compound (**6d**) gave 4-(2,4,6,-trichlorophenoxy)acetanilide as the main product and the yield of the expected compound (**7d**) was decreased. This side-reaction might proceed through an acid-catalysed cleavage of the N–O–C-1 linkage to give the corresponding amino derivative, followed by its acetylation.

Spectral Confirmation of The Products.—The structure of the orthoesters (**4a—d**) was confirmed by acid hydrolysis[15] to give the corresponding *N*-hydroxy-*N*-arylacetamides (**3a—d**) and by the spectral data. In the ^{1}H n.m.r. spectra of the compounds (**4a—d**) (Table 1), the resonance assignments were easily



Scheme 1. *Reagents:* i, 2,6-lutidine; ii, 2,6-lutidinium perchlorate



Scheme 2. *Reagents:* i, MeOH–Ba(OMe)$_2$ or MeOH–NH$_3$; ii, AcCl; iii, Ac$_2$O–pyridine

Table 1. Chemical shifts (δ_H) (100 MHz; $CDCl_3$) and coupling constants (J/Hz) of protons in the orthoesters (**4a**—**d**)

	Compound			
H Atom	(**4a**)	(**4b**)	(**4c**)	(**4d**)
1-H	5.59, d[a]	5.60 d	5.60, d	5.59, d
($J_{1,2}$)	(5.4)	(5.4)	(5.4)	(5.4)
2-H	4.28, dd	4.26, dd	4.26, dd	4.24, dd
($J_{2,3}$)	(3.2)	(3.0)	(3.2)	(3.2)
3-H	5.04, t	5.03, t	5.04, t	5.05, t
($J_{3,4}$)	(3.2)	(3.2)	(3.2)	(3.2)
4-H	4.84, dd	4.82, dd	4.82, dd	4.84, dd
($J_{4,5}$)	(9.3)	(9.4)	(9.4)	(9.4)
5-H	3.84, dt	3.84, dt	3.84, dt	3.84, dt
6-H	4.16, d	4.18, d	4.18, d	4.16, d
($J_{5,6}$)	(4.2)	(4.2)	(4.2)	(4.2)
OAc	2.08, s (3 H)	2.09, s (3 H)	2.09, s (3 H)	2.08, s (9 H)
	2.06, s (6 H)	2.08, s (6 H)	2.07, s (6 H)	
NAc	2.26, s (3 H)	2.27, s (3 H)	2.27, s (3 H)	2.24, s (3 H)
CMe	1.83, s (3 H)	1.84, s (3 H)	1.84, s (3 H)	1.82, s (3 H)
ArH[b]	7.22—7.41	7.26—7.36	7.45—7.52	7.41
	(5 H, m)	(4 H, m)	(2 H, m)	(2 H, s)
		6.92—7.01	7.35	7.36
		(4 H, m)	(1 H, d, J 2.4)	(2 H, d, J 9.0)
			6.86—7.27	6.81
			(4 H, m)	(2 H, d, J 9.0)

[a] Signal multiplicities: s, singlet; d, doublet; t, triplet; dd, double doublet; dt, double triplet; m, multiplet. [b] Protons of aromatic ring.

determined by a homonuclear decoupling experiment, as well as by comparison to the assignment of the t-butyl orthoester (**2**). The anomeric protons (1-H) of compounds (**4a**—**d**) resonated at δ 5.59—5.60 as doublets with J 5.4 Hz, a characteristic value[16] for bicyclic orthoesters of glucose having a *cis*-fused dioxolane ring. From the chemical shift of the methyl protons (C-Me) substituted on the dioxolane ring of the compounds (**4a**—**d**), it was proposed[17] that in all cases one of the two diastereoisomers, an *exo* orthoester isomer, was formed exclusively. In the e.i.–m.s. (electron-impact mass spectrum) of compound (**4a**), although its molecular ion peak was of low abundance, the ion with m/z 331, formed through elimination of *N*-phenyl-*N*-acetylnitroxyl radical, and secondary fragmentation ions at m/z 271, 211, 169, and 109, a characteristic pattern for acetylated glucosides,[18] were observed.

As shown in Table 2, in the ^{1}H n.m.r. spectra of the compounds (**5a**—**d**), the anomeric protons were observed at δ 4.86—4.88 as doublets with J 8.1—8.2 Hz, indicating[19] a β-configuration for compounds (**5a**—**d**). The β-configuration was further confirmed by the following results. (i) Catalytic hydrogenation[20] of compound (**5a**) gave acetanilide and 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranose, which indicated also that the compound (**5a**) has an N–O–C-1 linkage in the

Table 2. Chemical shifts (δ_H) (200 MHz; $CDCl_3$) and coupling constants (J/Hz) of protons in the glucosides (**5a**—**d**)

H Atom	Compound (**5a**)	(**5b**)	(**5c**)	(**5d**)
1-H	4.86, d[a]	4.86, d	4.88, d	4.88, d
($J_{1,2}$)	(8.1)	(8.1)	(8.2)	(8.1)
2-H, 3-H, 4-H	5.08—5.28, m	5.08—5.28, m	5.08—5.30, m	5.05—5.32, m
5-H	3.72, m	3.74, m	3.74, m	3.72, m
6-H	4.22, dd	4.23, dd	4.23, dd	4.24, dd
($J_{5,6}$)	(5.1)	(5.2)	(5.1)	(5.2)
($J_{6,6'}$)	(12.2)	(12.3)	(12.2)	(12.2)
6-H′	4.14, dd	4.15, dd	4.14, dd	4.14, dd
($J_{5,6}$)	(2.4)	(2.4)	(2.4)	(2.4)
($J_{6,6'}$)	(12.2)	(12.3)	(12.2)	(12.2)
OAc	2.00, s (3 H)	2.00, s (3 H)	2.00, s (6 H)	1.98, s (3 H)
	2.01, s (3 H)	2.01, s (3 H)	2.03, s (3 H)	2.00, s (3 H)
	2.02, s (3 H)	2.03, s (3 H)	2.09, s (3 H)	2.02, s (3 H)
	2.09, s (3 H)	2.08, s (3 H)		2.09, s (3 H)
NAc	2.29, s (3 H)	2.27, s (3 H)	2.27, s (3 H)	2.26, s (3 H)
ArH[b]	7.30—7.44	6.98	6.93	6.82
	(5 H, m)	(4 H, d, J 9.0)	(2 H, d, J 9.0)	(2 H, d, J 9.3)
		7.30	6.99	7.26
		(2 H, d, J 9.0)	(1 H, d, J 8.5)	(2 H, d, 9.3)
		7.33	7.25 (1 H, dd,	7.43, s (2 H)
		(2 H, d, J 9.0)	J 2.4 and 8.5)	
			7.29	
			(2 H, d, J 9.0)	
			7.49 (1 H, d, J	
			2.4)	

[a,b] See footnotes in Table 1.

molecule. (ii) Compounds (**5a**—**d**), prepared by the orthoester method, were identical with those obtained by the Koenigs–Knorr method. Both methods have been known to give a 1,2-*trans* glycoside. In the e.i.–m.s. spectrum of compound (**5a**), the molecular ion ($M^+ + 1$) was observed at m/z 482, together with fragmentation ions at m/z 331, 271, 211, 169, 127, and 109, a similar pattern to that of compound (**4a**).

The structure of the fully deacetylated compound (**6a**) and its derivative (**8a**) was confirmed by acetylation back to the parent compound (**5a**) as shown in Scheme 2. In the i.r. and ^1H n.m.r. spectra of compound (**6a**), no signal attributed to an acetyl group was observed and the u.v. spectrum resembled that of *N*-phenylhydroxylamine. Although the structure of compounds (**6b**—**d**) could not be confirmed because of their lability and difficulty of purification, compounds (**7b**—**d**), selectively *N*-acetylated derivatives of compounds (**6b**—**d**), were characterized by their spectral data. In the i.r. spectra of the monoacetates (**7a**—**d**), amide I bands were observed at 1 680—1 690 cm^{-1} and no absorption band attributed to an *O*-acetyl group was observed. The i.r. spectra of compounds (**3a**—**d**) exhibited amide I bands at 1 610—1 620 cm^{-1} owing to characteristic intramolecular hydrogen bonding,[21] whereas those of compounds (**5a**—**d**) and (**7a**—**d**) were observed at 1 680—1 690 cm^{-1}, which indicated that compounds (**5a**—**d**) and (**7a**—**d**) have an N–O–C-1 linkage in their molecules. The ^1H n.m.r. spectra of compounds (**5a**—**d**) showed singlets at δ 2.26—2.29 assigned to the methyl protons of the *N*-acetyl groups. This downfield shift by 0.2—0.3 p.p.m. compared with those of *N*-arylacetamides is thought to be characteristic for *N*-hydroxy-*N*-arylacetamide derivatives, because of the inductive effect of oxygen on the nitrogen atom. The β-configuration of these compounds [(**7a**—**d**)] was confirmed from the following: (i) J values[19] of the anomeric protons were 8.0—8.1 Hz, (ii) the negative value of the specific rotation[22] for compound (**7a**), (iii) these compounds were easily hydrolysed enzymatically to give the corresponding *N*-hydroxy-*N*-arylacetamides (**3a**—**d**) by β-glucosidase (emulsin) but not by α-glycosidase (yeast).

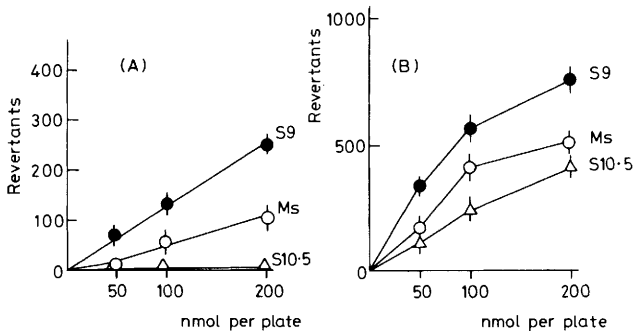


Figure 1. Mutagenic activity of the glucoside (**7b**) (A) and its aglycone (**3b**) (B) with intracellular fractions of guinea pig liver. Each point represents the means ± S.E. for at least 3 plates

Mutagenicity.—The mutagenic activity of compounds (**7a**—**d**) was tested in *Salmonella typhimurium* tester strain TA100. These compounds were non-mutagenic *per se*, but in the presence of guinea pig liver post-mitochondrial supernatant (S9) compounds (**7b**—**d**) exhibited mutagenic activity, although this activity was weak compared with that of the corresponding *N*-hydroxy-*N*-arylacetamides (**3b**—**d**) (data not shown). The order of mutagenic activity (a number of revertants, at 100 nmol of each chemical) was (**7b**) (130) > (**7c**) (50) > (**7d**) (35). The compounds having fewer chlorine atoms were more effective in inducing mutations than ones having multiple chlorines. The *O*-glucoside of *N*-hydroxyacetanilide, (**7a**), and of *N*-phenylhydroxylamine, (**6a**), were non-mutagenic as were *N*-phenylhydroxylamine and nitrosobenzene[23] at the concentrations tested (data not shown).

Several studies were undertaken to clarify the pathway of mutagenic activation of the glucosides (**7b**—**d**). Figure 1 shows the mutagenic activity of compound (**7b**) and its aglycone, *N*-hydroxy-4-(4-chlorophenoxy)acetanilide (**3b**), in the presence of intracellular fractions of guinea pig liver. Compound (**3b**) was also non-mutagenic *per se*, but it became mutagenic by metabolic activation with S9, microsomal fraction (Ms), and soluble supernatant fraction (S10.5). In the case of the glucoside (**7b**) it was noted that the mutagenic activation was caused by Ms and that no mutagenic activity was detected with S10.5.

Since deacetylase activity of guinea pig liver is known to be very high, the mutagenic product of an *N*-hydroxy-*N*-arylacetamide is thought to be the deacetylated form, namely the hydroxylamine derivative.[2,24] Mutagenic activation of the glucoside (**7b**) is also thought to be mediated by a microsomal deacetylase(s). Therefore, the effect of bis-(*p*-nitrophenyl) phosphate, an inhibitor of deacetylase, on the mutagenicity of compounds (**3b**) and (**7b**) was examined. As shown in Table 3, the mutagenic activity of both compounds was inhibited by the phosphate at a concentration of 10^{-3}M, though that of compound (**3b**) was not completely inhibited. The effect of the inhibitor on the mutagenicity of compound (**3b**) with S10.5 was less than that with Ms. From these data, it is suggested that the microsomal deacetylase(s) participates in mutagenic activation of the glucosides (**7b**—**d**). However, whether mutagenic activity of the glucosides (**7b**—**d**) results from initial cleavage of the compounds to their aglycones (**3b**—**d**) or not is not clear, although β-glucosidase activity capable of hydrolysing the glucoside bond of compounds (**7a**—**d**) might exist in Ms if it did.

Table 3. Effect of deacetylase inhibitor, bis-(*p*-nitrophenyl) phosphate, on the mutagenicity of compounds (**3b**) and (**7b**) by Ms or S10.5

Activating system	Concn of inhibitor (M)	Relative control activity (%) (**3b**)	(**7b**)
Ms	0	100	100
	10^{-5}	25.5	78.2
	10^{-4}	11.1	31.4
	10^{-3}	6.5	0.1
S10.5	0	100	
	10^{-5}	89.7	
	10^{-4}	41.7	
	10^{-3}	22.2	

[a] Mutagenic activity was determined by the number of revertants per plate and is expressed as percentage activity without modifiers for each substrate. The concentration of each substrate was 200 nmol per plate.

Table 4. Effect of several activating enzymes on the mutagenicity of compounds (**3b**) and (**7b**)

Activating system	Ratio[a] (**3b**)	(**7b**)
Ms	6.7 (6.8)[b]	1.6 (1.6)[b]
Ms + β-glucosidase	6.8 (6.8)[b]	4.6 (2.5)[b]
S10.5	3.6	1.0
S10.5 + β-glucosidase	3.7 (3.8)[b]	3.1 (1.8)[b]
β-Glucosidase	1.0	1.0
Carboxyesterase	8.9	1.0
Carboxyesterase + β-glucosidase	8.9 (9.0)[b]	7.7 (3.4)[b]

[a] Values in the Table are ratios of the number of revertants observed on the test plate (100 nmol per plate) to the number of revertants appearing on the corresponding control plate. [b] Values in parentheses are ratios in the presence of δ-gluconolactone (5×10^{-3}M).

Table 4 shows the mutagenic activity of compounds (**7b**) and (**3b**) in the presence of several combinations of activating enzymes including β-glucosidase (emulsin) and carboxyesterase (porcine liver). Addition of β-glucosidase to Ms resulted in enhancement of the mutagenic activity of the glucoside (**7b**), though β-glucosidase alone was unable to form mutagenic species. Use of carboxyesterase, as well as S10.5, each having no ability to activate the glucoside (**7b**), resulted in the appearance of a significant number of revertants when β-glucosidase was added. Addition of δ-gluconolactone, a β-glucosidase inhibitor, had no influence on the activating capacity of Ms but caused *ca.* 40% inhibition of the activating capacity associated with β-glucosidase.

To validate the pathway of mutagenic activation of the glucosides (**7b**—**d**), the glucoside (**7a**), a non-mutagenic compound, was incubated with Ms and the metabolites were analysed by high-performance liquid chromatography (h.p.l.c.). The glucoside (**7a**) was chosen because possible metabolites including the corresponding *N*-deacetylated compound (**6a**) have been prepared.

As shown in Figure 2, the corresponding *N*-deacetylated compound (**6a**) could be detected as the main metabolite, which gradually decomposed to give aniline and a smaller amount of azoxybenzene through a non-enzymatic process. When bis-(*p*-nitrophenyl) phosphate was added to the incubation mixture after 30 min at a concentration of 10^{-3}M, the *N*-deacetylation of the glucoside (**7a**) was completely inhibited and no formation of the corresponding aglycone (**3a**) was observed. When the glucoside (**7a**) was incubated with carboxyesterase, no

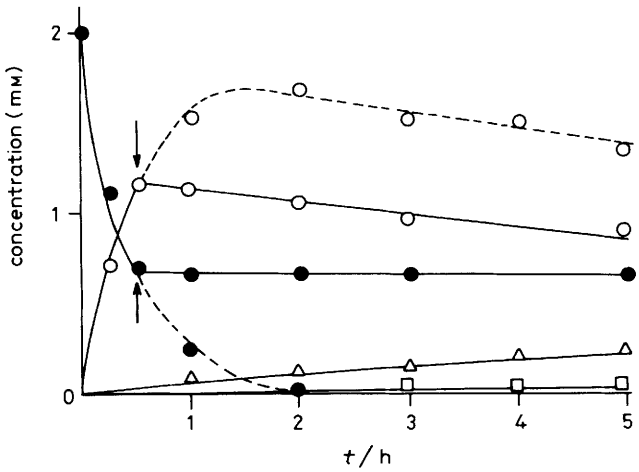


Figure 2. H.p.l.c. analysis of the glucoside (**7a**) by Ms. After 30 min (indicated as arrow), bis-(*p*-nitrophenyl) phosphate (final concn, 10^{-3}M) was used as an inhibitor. (●) Glucoside (**7a**), (○) glucoside (**6a**), (△) aniline, (□) azoxybenzene. Dashed lines: without addition of the inhibitor

enzymatic or chemical conversion products were detected until after 3 h by h.p.l.c. analysis. These results suggest that directly acting mutagens of the glucosides (**7b**—**d**) are the *N*-deacetylated compounds (**6b**—**d**) formed by the microsomal deacetylase(s), different from the cytosolic deacetylase(s).

Discussion

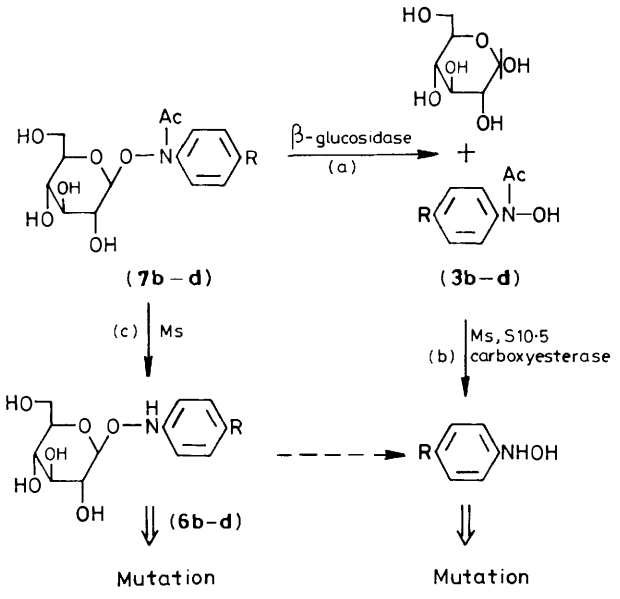
The first *O*-glycoside of an *N*-hydroxy-*N*-arylacetamide was isolated [25] from the urine of rabbits fed 2-(acetamido)fluorene and was found to be the *O*-glucuronide of *N*-hydroxy-2-(acetamido)fluorene; the structure of the product was confirmed by its conversion into the methyl acetyl derivative. The first chemical synthesis of methyl acetyl derivatives of these glucuronides was achieved by Irving [25] and Fishman [26] using the Koenigs–Knorr method, though their yields were poor. Furthermore, deprotection of the compounds to the *O*-glucuronides of *N*-hydroxy-*N*-arylacetamides was unsuccessful. These *O*-glucuronides have been obtained only biosynthetically, by both *in vivo* [3–5] and *in vitro*.[6–8] methods. However, their chemical and biological characteristics have not been fully investigated possibly due in part to limited availability.

The orthoester method has become increasingly significant for the synthesis of saccharides,[27] β-D-glucopyranosyl phosphate,[28] and other esters.[29] The application of this method to the synthesis of compounds (**5a**—**d**) resulted in better yields than those obtained by the Koenigs–Knorr method. In the Koenigs–Knorr reaction, *N*-hydroxy-*N*-arylacetamides (**3a**—**d**) were decomposed to a complicated mixture through a radical-mediated degradation reaction,[30] and for this reason the yields obtained by the Koenigs–Knorr method are poor.

Selective *O*-deacetylation of compounds (**5a**—**d**) to compounds (**7a**—**d**) was unsuccessful because hydrolysis of both the *O*- and *N*-acetyl groups was followed by cleavage of the glucoside bond. Therefore, synthesis of the glucosides (**7a**—**d**) was achieved by deacetylation of all the acetyl groups of compounds (**5a**—**d**) followed by selective *N*-re-acetylation. Chemical synthesis and characterization of the *O*-glycosides of *N*-hydroxy-*N*-arylamines have not previously been reported except for the *O*-glucuronide of *N*-hydroxyfluoren-2-ylamine,[4] though the structure has not been fully confirmed by the spectral data. The structure of compound (**6a**) could be confirmed by its spectral data and this free amine was found to

decompose to aniline, with concomitant formation of D-gluconic acid and a smaller amount of azoxybenzene, in neutral aqueous solution. These redox and hydrolytical cleavages of the N–O–C-1 linkage of the glucoside (**6a**) will be discussed in a subsequent paper.[31]

In considering the mutagenic activation of the glucosides (**7b—d**), the possibility that hydrolysis of the glucosides to their aglycones (**3b—d**) in the presence of Ms was occurring prior to enzymatic deacetylation was not supported for the following reasons: (i) mutagenic activity of the glucoside (**7b**) caused by Ms was not affected by δ-gluconolactone, a β-glucosidase inhibitor (Table 4), (ii) when the glucoside (**7a**) was incubated with Ms in the presence of bis-(*p*-nitrophenyl) phosphate, a deacetylase inhibitor, the aglycone (**3a**) was not detected by h.p.l.c. analysis (Figure 2) and the inhibitor caused a decrease in the mutagenic activity of the glucoside (**7b**) (Table 3). It is concluded that guinea pig liver has no β-glucosidase activity capable of hydrolysing the glucosides (**7a—d**), though a non-specific β-glucosidase has been reported to be present in the mammal's tissues.[32] The mutagenic pathway of the glucosides (**7b—d**) is summarized in Scheme 3. In the presence of β-glucosidase, the directly acting mutagen is the corresponding hydroxylamino derivatives formed *via* paths (a) and (b), through the corresponding intermediate acetohydroxamic acids (**3a—d**). In the mutagenic activation of the glucosides (**7b—d**) caused by Ms, the directly acting mutagen seemed to be the corresponding *N*-deacetylated compounds (**6b—d**) formed *via* path (c). The inability of S10.5 to activate the mutagenicity of the glucosides (**7b—d**) is attributed to its complete lack of, or weak, *N*-deacetylating activity. In an indirect mutagenic assay, Bos *et al.* reported[33,34] that *O*-glucuronides of *N*-hydroxy-*N*-arylacetamides exhibited no mutagenic activity in the presence of rat, mouse, and guinea pig liver post-microsomal supernatant, which indicated the possibility that rat and mouse S10.5 also have little or no *N*-deacetylating activity. Therefore, the microsomal deacetylase(s) of guinea pig liver is different from the cytosolic deacetylase(s) and porcine liver carboxyesterase with regard to the ability to *N*-deacetylate the glucosides (**7a—d**). The *O*-sulphates and the *O*-glucuronides of *N*-hydroxy-*N*-arylacetamides and *N*-acyloxy-*N*-arylamines are reported to be the ultimate carcinogens[15] which are highly mutagenic in their ability to transform DNA.[35] However, the *O*-sulphates of *N*-hydroxy-*N*-arylacetamide are reported[36] to be weakly mutagenic in the *Salmonella* test system. The test seemed to be unable to detect short lived highly reactive mutagens such as the *O*-sulphates of *N*-hydroxy-*N*-arylacetamides because they were easily trapped by interaction with nucleophiles and nucleophilic sites of other macromolecules prior to interaction with bacterial DNA. This might be the reason why the mutagenic activity of the highly reactive *N*-deacetylated compounds (**6b—d**), formed from the less reactive compounds (**7b—d**), as well as the *O*-glucuronides of *N*-hydroxy-*N*-arylamines,[37] was weak. Of the glucosides (**7b—d**), the differences in their mutagenicity would be due to the mutagenic activity of the corresponding *N*-deacetylated compounds (**6b—d**) by themselves and/or the sensitivity of the glucosides (**7b—d**) to the microsomal deacetylase(s). The electrophilicity and mutagenicity of compounds (**6b—d**) formed from the glucosides (**7b—d**) by microsomal deacetylase(s), and the nature of the enzyme(s), will be further investigated.



Scheme 3. Mutagenic pathway of the glucosides (**7a—d**)

Experimental

M.p.s were determined by the capillary method and are uncorrected. I.r. spectra were recorded on a JASCO A-102 spectrophotometer as either solutions in chloroform or KBr discs. U.v. spectra were recorded on a Shimadzu UV-200S spectrophotometer. ^{1}H N.m.r. spectra were recorded on JEOL JNM-FX100 or -FX200 spectrometers as solutions in either $(CD_3)_2SO$ or $CDCl_3$, using Me_4Si as internal standard. Optical rotations were measured on a JASCO Dip-4 digital polarimeter using a 5 cm path-length cell. Electron-impact mass spectra were recorded on a Shimadzu-LKB 9000B instrument by using a direct-inlet system. T.l.c. was performed on Kieselgel $60F_{254}$ (Merck 5554) and for column chromatography Kieselgel (Merck 7734) was used.

General Procedure for the Preparation of N-*Hydroxy*-N-*arylacetamides* (**3a—d**).—To a stirred solution of a nitroaromatic compound $RC_6H_4NO_2$-*p* (0.1 mol) in dioxane (200 ml), except for the case of nitrobenzene in 50% ethanol, and 2M-aqueous ammonium chloride (50 ml) was added in portions zinc dust (0.25 g-atom) during 10 min while the reaction mixture was kept at 50—60 °C. The resultant mixture was stirred for an additional 15 min. After the mixture had been filtered through a Celite bed, the filtrate was evaporated under reduced pressure to small volume and the residue was extracted with ether (4 × 50 ml). To the combined ether extracts were added sodium hydrogen carbonate (0.3 mol) and water (100 ml). A solution of acetyl chloride (0.21 mol) in ether (30 ml) was then added dropwise to the vigorously stirred mixture in an ice-bath. After 1.5 h, the organic phase was diluted with n-hexane (100 ml), stirred, and cold 1M-aqueous sodium hydroxide saturated with sodium chloride was added. The precipitate, a sodium salt, was suspended in ether (200 ml) and the mixture was neutralized with 2M-aqueous potassium phosphate (monobasic) (200 ml). The ether layer was washed with water, dried (Na_2SO_4), and evaporated under reduced pressure to give the corresponding *N*-hydroxy-*N*-arylacetamide (**3a—d**). Yields and m.p.s were as follow. *N*-Hydroxyacetanilide (**3a**), 65%, 67 °C (from ethyl acetate–n-hexane) (lit.,[38] 67—67.5 °C).

4-(4-*Chlorophenoxy*)-N-*hydroxyacetanilide* (**3b**), 64%, 103—104 °C (from benzene–n-hexane).

4-(2,4-*Dichlorophenoxy*)-N-*hydroxyacetanilide* (**3c**), 58%, 115—115.5 °C (from benzene–n-hexane).

N-*Hydroxy*-4-(2,4,6-*trichlorophenoxy*)*acetanilide* (**3d**), 65%, 130—130.5 °C (from benzene–n-hexane). Spectral data and elemental analyses of these compounds are shown in Table 5.

Table 5. Analytical data of *N*-hydroxy-*N*-arylacetamides (**3b—d**)

Compound (Formula)	Analysis (%)[a] C	H	N	$\lambda_{max.}$(EtOH) (nm)	$\nu_{max.}$(KBr) (cm^{-1})	δ_H[$(CD_3)_2SO$]	*m/z*
(**3b**)	60.7	4.3	4.9	258.5	3 130	10.63 (1 H, s)	277 (M^+)
($C_{14}H_{12}ClNO_3$)	(60.54)	(4.32)	(5.05)	(ε 12 600)	1 620	7.62 (2 H, d, *J* 9.2 Hz)	261 ($M^+ - 16$)[b]
					1 595	7.42 (2 H, d, *J* 9.2 Hz)	235 ($M^+ - 42$)
					1 505	7.04 (2 H, d, *J* 9.2 Hz)	219 (base)
					1 240	7.01 (2 H, d, *J* 9.2 Hz)	
						2.19 (3 H, s)	
(**3c**)	53.8	3.4	4.2	258.5	3 160	10.64 (1 H, s)	311 (M^+)
($C_{14}H_{11}Cl_2NO_3$)	(53.85)	(3.52)	(4.49)	(ε 12 800)	1 610	7.76 (1 H, d, *J* 2.4 Hz)	295 ($M^+ - 16$)[b]
					1 595	7.62 (2 H, d, *J* 9.0 Hz)	269 ($M^+ - 42$)
					1 260	7.43 (1 H, dd, *J* 2.4 and 8.8 Hz)	253 (base)
						7.08 (1 H, d, *J* 8.8 Hz)	
						7.00 (2 H, d, *J* 9.0 Hz)	
						2.19 (3 H, s)	
(**3d**)	48.5	2.8	3.9	256	3 150	10.60 (1 H, s)	345 (M^+)
($C_{14}H_{10}Cl_3NO_3$)	(48.48)	(2.89)	(4.04)	(ε 9 800)	1 610	7.88 (2 H, s)	329 ($M^+ - 16$)[b]
					1 595	7.54 (2 H, d, *J* 9.0 Hz)	303 ($M^+ - 42$)
					1 260	6.86 (2 H, d, *J* 9.0 Hz)	287 (base)
						2.17 (3 H, s)	

[a] Required values in parentheses. [b] J. A. Hinson, J. R. Mitchell, and D. J. Jollow, *Mol. Pharmacol.*, 1975, **20**, 1178.

General Procedure for the Preparation of the Orthoesters (**4a—d**).—To a solution of 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide (**1**) (2 mmol) and the respective *N*-hydroxy-*N*-arylacetamide (**3a—d**) (2 mmol) in nitromethane (1 ml) was added 2,6-lutidine (0.5 ml, 4.3 mmol) and the resultant mixture was kept at 37 °C in the dark. After 42 h, except for the case of compound (**3a**) (30 h), ether (30 ml) was then added and the mixture was filtered to remove 2,6-lutidinium bromide. The filtrate was diluted with ether (15 ml) and washed with water, dried (Na_2SO_4), and evaporated under reduced pressure. In the case of compound (**3a**), the residue was triturated with ether to give a colourless solid, which was purified by recrystallization. In the other cases, purification was achieved by silica gel column chromatography with 1:3 ethyl acetate–benzene as eluant. Physical constants for the orthoesters (**4a—d**) are given below. ^{1}H N.m.r. spectra are shown in Table 1. 3,4,6-*Tri*-O-*acetyl*-1,2-O-[1-(*N*-*acetylanilino-oxy*)*ethylidene*]-α-D-*glucopyranose* (**4a**), 85%, m.p. 132.5—133.5 °C (from ether–n-hexane) (Found: C, 55.0; H, 5.7; N, 2.8. $C_{22}H_{27}NO_{11}$ requires C, 54.88; H, 5.65; N, 2.91%); $\nu_{max.}$(KBr) 1 740, 1 695, 1 600, and 1 280 cm^{-1}; $\lambda_{max.}$(EtOH) 240 nm (ε 7 250); *m/z* 481 (M^+), 331, 271, 211, 169 (base peak), and 109 (99%).

3,4,6-*Tri*-O-*acetyl*-1,2-O-{1-[*N*-*acetyl*-4-(4-*chlorophenoxy*)-*anilino-oxy*]*ethylidene*}-α-D-*glucopyranose* (**4b**), 80%, amorphous (Found: C, 55.4; H, 5.0; N, 2.3; Cl, 5.9. $C_{28}H_{30}ClO_{12}N$ requires C, 55.31; H, 4.97; N, 2.30; Cl, 5.83%); $\nu_{max.}$($CHCl_3$) 1 750, 1 690, 1 595, and 1 220 cm^{-1}; $\lambda_{max.}$(EtOH) 249 nm (ε 13 400).

3,4,6-*Tri*-O-*acetyl*-1,2-O-{1-[*N*-*acetyl*-4-(2,4-*dichloro-phenoxy*)*anilino-oxy*]*ethylidene*}-α-D-*glucopyranose* (**4c**), 80%, amorphous (Found: C, 52.2; H, 4.6; N, 2.0; Cl, 11.0. $C_{28}H_{29}Cl_2NO_{12}$ requires C, 52.35; H, 4.55; N, 2.18; Cl, 11.04%); $\nu_{max.}$($CHCl_3$) 1 754, 1 690, 1 600, and 1 230 cm^{-1}; $\lambda_{max.}$(EtOH) 247 nm (ε 14 100).

3,4,6-*Tri*-O-*acetyl*-1,2-O-{1-[*N*-*acetyl*-4-(2,4,6-*trichloro-phenoxy*)*anilino-oxy*]*ethylidene*}-α-D-*glucopyranose* (**4d**), 84%, amorphous (Found: C, 49.7; H, 4.2; N, 2.0. $C_{28}H_{28}Cl_3NO_{12}$ requires C, 49.68; H, 4.17; N, 2.07%); $\nu_{max.}$($CHCl_3$) 1 745, 1 690, 1 505, and 1 240 cm^{-1}; $\lambda_{max.}$(EtOH) 244 (ε 10 100) and 226sh nm (11 850).

General Procedure for the Rearrangement of the Orthoesters (**4a—d**) *into Tetra*-O-*acetyl*-β-D-*glucosides* (**5a—d**).—A solution of an orthoester (**4a—d**) (10 mmol) in anhydrous ethylene dichloride (150 ml) was refluxed in the presence of a catalytic amount of 2,6-lutidinium perchlorate (100 mg, 48 μmol). Reaction time in the case of compound (**4a**), **4b**), (**4c**), and (**4d**) was 12, 25, 25, and 26 h respectively. The solvent was then removed under reduced pressure and the residue was chromatographed on silica gel with 1:4 ethyl acetate–benzene as eluant to give the corresponding compound (**5a—d**). The spectral data except for the ^{1}H n.m.r. spectra (shown in Table 2) are given below. Thus prepared were N-*acetylanilino* 2,3,4,6-*tetra*-O-*acetyl*-β-D-*glucopyranoside* (**5a**), 59%, m.p. 109—110 °C (from ether–n-hexane) (Found: C, 54.85; H, 5.8; N, 2.9. $C_{22}H_{27}NO_{11}$ requires C, 54.88; H, 5.65; N, 2.91%); $[\alpha]_D^{11}$ −34.8° (*c* 1 in EtOH); $\nu_{max.}$(KBr) 1 740, 1 680, 1 590, and 900 cm^{-1}; $\lambda_{max.}$(EtOH) 240 nm (ε 6 700).

N-*Acetyl*-4-(4-*chlorophenoxy*)*anilino* 2,3,4,6-*tetra*-O-*acetyl*-β-D-*glucopyranoside* (**5b**), 35%, m.p. 136—138 °C (from ether–n-hexane) (Found: C, 55.2; H, 5.0; N, 2.4. $C_{28}H_{30}ClNO_{12}$ requires C, 55.31; H, 4.97; N, 2.30%); $[\alpha]_D^{11}$ −28.3° (*c* 0.92 in EtOH); $\nu_{max.}$(KBr) 1 755, 1 690, 1 590, 1 240, and 910 cm^{-1}; $\lambda_{max.}$(EtOH) 247 nm (ε 13 500).

N-*Acetyl*-4-(2,4-*dichlorophenoxy*)*anilino* 2,3,4,6-*tetra*-O-*acetyl*-β-D-*glucopyranoside* (**5c**), 29%, m.p. 128.5—129.5 °C (from ether–n-hexane) (Found: C, 52.35, H, 4.45; N, 2.3; Cl, 11.0. $C_{28}H_{29}Cl_2NO_{12}$ requires C, 52.35; H, 4.55; N, 2.18; Cl, 11.04%); $[\alpha]_D^{11}$ −6.3° (*c* 0.96 in EtOH); $\nu_{max.}$(KBr) 1 745, 1 680, 1 500, 1 230, and 905 cm^{-1}; $\lambda_{max.}$(EtOH) 243 nm (ε 10 100).

N-*Acetyl*-4-(2,4,6-*trichlorophenoxy*)*anilino* 2,3,4,6-*tetra*-O-*acetyl*-β-D-*glucopyranoside* (**5d**), 30%, m.p. 104—106 °C (from ether–n-hexane) (Found: C, 49.9; H, 4.4; N, 1.9; Cl, 15.1. $C_{28}H_{28}Cl_3NO_{12}$ requires C, 49.68; H, 4.17; N, 2.07; Cl, 15.71%); $[\alpha]_D^{11}$ −5.0° (*c* 0.8 in EtOH); $\nu_{max.}$(KBr) 1 760, 1 690, 1 560, 1 230, and 910 cm^{-1}; $\lambda_{max.}$ (EtOH) 285sh (ε 1 100) and 235sh nm (12 500).

Hydrogenolysis of Compound (**5a**).—Compound (**5a**) (160 mg, 0.33 mmol) in EtOH (20 ml) was hydrogenolysed under hydrogen (1 atm) in the presence of 10% palladium–charcoal

catalyst (20 mg) for 2 h. Filtration and evaporation of the filtrate gave a mixture of two products, separated by preparative t.l.c. (p.l.c.), which proved to be acetanilide (42 mg, 94%) (m.p. and mixed m.p. 114—115 °C) and 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranose[39] (110 mg, 96%) (m.p. and mixed m.p. 132—134 °C).

Synthesis of O-Glucosides (**5a—d**) *by the Koenigs–Knorr Method.*—To a solution of an *N*-hydroxy-*N*-arylacetamide (**3a—d**) (1 mmol) and compound (**1**) (2 mmol) in benzene (15 ml) was added silver(I) carbonate (2.5 mmol) and the mixture was vigorously stirred at room temperature for 24 h. During the reaction, the flask was wrapped with tin-foil to protect it from light. After filtration, the filtrate was evaporated under reduced pressure and the residue was chromatographed on silica gel with 1:3 ethyl acetate–benzene as eluant. The fraction containing the product was then purified by p.l.c. to give the corresponding *O*-glucoside (**5a—d**). The yield of compound (**5a**), (**5b**), (**5c**), and (**5d**) was 21, 14, 16, and 5%, respectively.

Anilino β-D-*Glucopyranoside* (**6a**).—*Method A.* An ice-cooled solution of compound (**5a**) (720 mg, 1.5 mmol) in anhydrous methanol (3 ml) was saturated with ammonia. After 2 h, the reaction mixture was evaporated under reduced pressure and the residue was triturated with ether (5 ml) to give compound (**6a**) (335 mg, 71.4%).

Method B. To a solution of compound (**5a**) (960 mg, 2 mmol) in anhydrous methanol (6 ml), cooled in an ice-bath, was added 0.1M-methanolic barium methoxide (2 ml, 0.2 mmol). After 1.5 h, 1M-aqueous ammonium sulphate (0.2 ml) was added and the mixture was filtered. The filtrate was evaporated under reduced pressure and the residue was triturated with ether (20 ml) to give compound (**6a**) (495 mg, 79%). Purification was achieved by recrystallization from ethyl acetate, m.p. 110 °C (decomp.) (Found: C, 52.6; H, 6.5; N, 5.1. $C_{12}H_{17}NO_6$ requires C, 53.13; H, 6.32; N, 5.16%); $[\alpha]_D^{19}$ −64.5° (*c* 1 in water); $\nu_{max.}$(KBr) 3 300, 1 610, 1 500, and 1 080 cm^{-1}; $\lambda_{max.}$(water) 231 (ε 8 600) and 273 nm (900); δ_H [100 MHz; $(CD_3)_2SO$] 9.24 (1 H, s, exchangeable with D_2O) and 6.80—7.28 (5 H, m). Other protons including the anomeric proton were not further characterized. However, there was no resonance in the range δ_H 2.00—2.30.

Acetylation of Compound (**6a**) *with Pyridine–*Ac_2O.—To a stirred solution of compound (**6a**) (138 mg, 0.51 mmol) in pyridine (1 ml) was added Ac_2O (0.3 ml, 3 mmol) and the solution was kept at 0 °C. After the reaction was complete (2 h), the reaction mixture was poured onto ice–water and then extracted with ether (2 × 10 ml). The combined ether layer was washed successively with 0.1M-HCl, water, and saturated aqueous $NaHCO_3$ and was then dried (Na_2SO_4). The solvent was evaporated under reduced pressure, and the residue was purified by p.l.c. to give the per-acetylated glucoside (**5a**) (38 mg, 16%), identical with the authentic compound, and unstable compound (**8a**) (99 mg, 62%), $\nu_{max.}$(KBr) 3 300, 1 755, 1 600, 1 370, 1 220, and 1 040 cm^{-1}; δ_H[$(CD_3)_2SO$] 9.60 (1 H, br s, exchangeable with D_2O), 7.16—7.32 (2 H, m), 6.82—6.96 (3 H, m), 4.84–5.50 (4 H, m), 4.08—4.18 (3 H, m), 2.10 (3 H, s), 2.03 (3 H, s), 2.00 (3 H, s), and 1.98 (3 H, s). Compound (**8a**) (40 mg) was again acetylated by pyridine (0.5 ml) and Ac_2O (0.15 ml 1.5 mmol) to give the per-acetylated glucoside (**5a**) (36 mg, 82%).

N-*Acetylanilino* β-D-*Glucopyranoside* (**7a**).—To a solution of compound (**6a**) (378 mg, 1.4 mmol) in dioxane (40 ml) were added sodium hydrogen carbonate (235 mg, 2.8 mmol) and powdered 4Å-molecular sieves (500 mg), and then acetyl chloride (0.125 ml, 1.7 mmol) was added in one portion. After the mixture had been stirred at room temperature for 1.5 h, ethanol (0.05 ml, 0.8 mmol) was added and the mixture was stirred for an additional 15 min. Filtration and evaporation of the filtrate gave a syrupy residue which was chromatographed on silica gel with chloroform–acetone–water (4:18:1) as eluant to give the *title compound* (**7a**) (275 mg, 63%), m.p. 104—105 °C (from acetonitrile) (Found: C, 51.5; H, 6.1; N, 4.2. $C_{14}H_{19}O_7N\cdot\frac{1}{2}H_2O$ requires C, 52.17; H, 6.25; N, 4.35%); $[\alpha]_D^{11}$ −54.0° (*c* 0.6 in water); $\nu_{max.}$(KBr) 3 350, 2 950, 1 680, 1 590, 1 490, 1 380, and 1 080 cm^{-1}; $\lambda_{max.}$(water) 235 nm (ε 4 800); δ_H [100 MHz; $(CD_3)_2SO$–D_2O] 2.29 (3 H, s), 3.18 (4 H, br s), 3.34—3.72 (2 H, m), 4.54 (1 H, d, *J* 8.0 Hz, 1-H), and 7.22—7.64 (5 H, m).

N-*Acetyl*-4-(4-*chlorophenoxy*)*anilino* β-D-*Glucopyranoside* (**7b**).—Compound (**5b**) (486 mg, 0.8 mmol) in anhydrous methanol (4 ml) was deacetylated with 0.1M-methanolic barium methoxide (1.6 ml, 0.16 mmol) according to method B (described above). The deacetylated product (**6b**) (200 mg, 63%) was then acetylated without further purification. Thus, to a solution of compound (**6b**) in dioxane (10 ml) were added sodium hydrogen carbonate (84 mg, 1 mmol) powdered 4Å-molecular sieves (350 mg), and triethylamine (0.14 ml, 1 mmol) and then acetyl chloride (40 μl, 0.54 mmol). After being stirred for 2 h, the reaction mixture was filtered and the filtrate was evaporated under reduced pressure. The residue was chromatographed on silica gel with chloroform–methanol (10:2) as eluant to give the *title compound* (**7b**) (140 mg, overall yield 40%), m.p. 116—118 °C (from acetonitrile) (Found: C, 53.4; H, 4.9; N, 3.1. $C_{20}H_{22}ClNO_8\cdot\frac{1}{2}H_2O$ requires C, 53.52; H, 5.17; N, 3.12%); $\nu_{max.}$(KBr) 3 350, 2 950, 1 680, 1 505, 1 490, 1 250, and 1 085 cm^{-1}; $\lambda_{max.}$(water) 243 nm (ε 14 800); δ_H [100 MHz; $(CD_3)_2SO$–D_2O] 2.28 (3 H, s), 3.14 (4 H, br s), 3.36—3.78 (2 H, m), 4.54 (1 H, d, *J* 8.1 Hz, 1-H), 7.02 (2 H, d, *J* 9.0 Hz), 7.04 (2 H, d, *J* 9.0 Hz), 7.44 (2 H, d, *J* 9.0 Hz), and 7.54 (2 H, d, *J* 9.0 Hz).

N-*Acetyl*-4-(2,4-*dichlorophenoxy*)*anilino* β-D-*Glucopyranoside* (**7c**).—Compound (**5c**) (642 mg, 1 mmol) in anhydrous methanol (5 ml) was deacetylated with 0.1M-methanolic barium methoxide (2 ml, 0.2 mmol). The deacetylated product (**6c**) (405 mg, 94%) was then acetylated without further purification. Thus, to a solution of compound (**6c**) in dioxane (15 ml) were added powdered 4Å-molecular sieves (400 mg), sodium hydrogen carbonate (151 mg, 1.8 mmol), and triethylamine (0.28 ml, 2 mmol), and then acetyl chloride (81 μl, 1.08 mmol). After being stirred for 2.5 h, the reaction mixture was filtered and the filtrate was evaporated under reduced pressure. The residue was chromatographed on silica gel with chloroform–methanol (10:2) as eluant to give the *title compound* (**7c**) (243 mg, overall yield 51%), m.p. 129.5—131 °C (from acetonitrile) (Found: C, 49.5; H, 4.5; N, 3.0. $C_{20}H_{21}Cl_2NO_8\cdot\frac{1}{2}H_2O$ requires C, 49.70; H, 4.59; N, 2.90%); $\nu_{max.}$(KBr) 3 350, 2 950, 1 680, 1 500, 1 380, 1 260, and 1 080 cm^{-1}; $\lambda_{max.}$(water) 239 nm (ε 11 000); δ_H [100 MHz; $(CD_3)_2SO$–D_2O] 2.28 (3 H, s), 3.18 (4 H, br s), 3.38—3.76 (2 H, m), 4.54 (1 H, d, *J* 8.0 Hz, 1-H), 6.98 (2 H, d, *J* 9.0 Hz), 7.15 (1 H, d, *J* 8.5 Hz), 7.46 (1 H, dd, *J* 2.4 and 8.5 Hz), 7.55 (2 H, d, *J* 9.0 Hz), and 7.78 (1 H, d, *J* 2.4 Hz).

N-*Acetyl*-4-(2,4,6-*trichlorophenoxy*)*anilino* β-D-*Glucopyranoside* (**7d**).—Compound (**5d**) (343 mg, 0.54 mmol) in anhydrous methanol (4 ml) was deacetylated with 0.1M-methanolic barium methoxide (1 ml, 0.1 mmol). The deacetylated product (**6d**) (230 mg, 91%) was then acetylated without further purification. Thus, to a solution of compound (**6d**) in dioxane (10 ml) were added sodium hydrogen carbonate (84 mg, 1 mmol), powdered 4Å-molecular sieves (250 mg), and triethylamine (0.14 ml, 1 mmol). After the addition of acetyl chloride (45 μl, 0.6 mmol), the reaction mixture was stirred for 2.5 h. Filtration and evaporation of the filtrate gave a residue which was chromatographed on silica gel with chloroform–

methanol (10:2) as eluant to give the *title compound* (**7d**) (146 mg, overall yield 53%), m.p. 161—162 °C (from acetonitrile) (Found: C, 45.85; H, 4.1; N, 2.7. $C_{20}H_{20}Cl_3NO_8 \cdot H_2O$ requires C, 45.60; H, 4.21; N, 2.66%); $\nu_{max.}$(KBr) 3 400, 2 950, 1 690, 1 505, 1 445, 1 260, and 1 080 cm^{-1}; $\lambda_{max.}$(water) 227sh (ε 12 000) and 235sh nm (10 500); δ_H [100 MHz; $(CD_3)_2SO$–D_2O] 2.27 (3 H, s), 3.14 (4 H, br s), 3.35—3.78 (2 H, m), 4.56 (1 H, d, *J* 8.0 Hz), 6.86 (2 H, d *J* 9.0 Hz), 7.50 (2 H, d, *J* 9.0 Hz), and 7.88 (2 H, s).

Without the addition of triethylamine, compound (**6d**) (230 mg, 0.49 mmol) gave the corresponding *N*-acetylated glucoside (**7d**) (80 mg, 32%) and 4-(2,4,6-*trichlorophenoxy*)*acetanilide* (100 mg, 61%), m.p. 209—210 °C (from ethyl acetate–n-hexane) (Found: C, 50.7; H, 3.1; N, 4.15. $C_{14}H_{10}Cl_3NO_2$ requires C, 50.86; H, 3.05; N, 4.24%); $\nu_{max.}$(KBr) 3 275, 1 655, 1 445, and 1 260 cm^{-1}; δ_H [$(CD_3)_2SO$] 2.02 (3 H, s), 6.79 (2 H, d, *J* 9.0 Hz), 7.54 (2 H, d, *J* 9.0 Hz), 7.86 (2 H, s), and 9.92 (1 H, br s).

Enzymatic Hydrolysis of the O-*Glucosides* (**7a—d**).—A 1.0 ml solution of an *O*-glucoside (**7a—d**) (final concentration 1.26 mM) in 0.1M-aqueous sodium acetate buffer (pH 5.5) was pre-incubated at 37 °C for 5 min. After the addition of β-glucosidase (50 μl) (emulsin, from Sigma; 3.4 units), the solution was incubated for an additional 30 min. The liberated aglycones, *N*-hydroxy-*N*-arylacetamides (**3a—d**), were extracted with ether. They showed the same R_F values on a silica gel t.l.c. plate with the respective authentic compounds and gave positive responses to $FeCl_3$ spray reagent. In both cases (i) and (ii), (i) without the enzyme and (ii) with α-glucosidase (from Sigma, 3 units; and for this case 0.1M-aqueous phosphate buffer, pH 6.9, was used), there was no liberation of the *N*-hydroxy-*N*-arylacetamides (**3a—d**).

Bacterial Mutagenicity Analysis.—Reversion to prototrophy using *Salmonella typhimurium* histidine auxotrophic strain TA100 was measured according to the Ames method[40] as modified by Yahagi.[41] The strain TA100 was used for the assay of mutagenicity because chlorinated 4-nitrobiphenyl ethers were classified as base-change mutagens.[42] S9 was prepared from PCB-pretreated male guinea pigs as described previously.[42] Subfractionation of the preparation was by centrifugation at 105 000 g for 1 h. A microsomal pellet was suspended in 0.15M-aqueous KCl (equal volume to the original S9) and centrifuged at 105 000 g for 1 h. The supernatant was decanted and the microsomal fraction (Ms) was resuspended in 0.15M-aqueous KCl of equal volume to the original S9. For the observation of deacetylase inhibition, bis-(*p*-nitrophenyl) phosphate at varying concentration (10^{-5}M to 10^{-3}M) was used. The β-glucosidase inhibitor, δ-gluconolactone, was used at the concentration of 5×10^{-3}M. On preincubation of the substrate with β-glucosidase (Sigma; 1 mg per plate), 10 mM-aqueous phosphate buffer (pH 6.0) in a total volume of 0.3 ml was used. After incubation, 0.1M-aqueous phosphate buffer (pH 7.4) (0.2 ml) was added and then followed by the standard pour-plate method. Carboxyesterase (Sigma, porcine liver type I; 5 units per plate) was used under standard conditions (pH 7.4). The mutagenic activity of the compounds was represented as the mean ± S.E. of a number of revertants of at least three plates. 4-Nitroquinoline 1-oxide (0.5 μg per plate) was used as a positive control for the bacteria.

High-performance Liquid Chromatography (*H.p.l.c.*) *Analysis.*—The incubation mixture, in a test tube, consisted of DMSO (0.2 ml) containing the glucoside (**7a**) (2.8 μmol), 0.1M-aqueous phosphate buffer (pH 7.4) (0.9 ml), and Ms (0.3 ml). Incubations were run at 37 °C in a shaken water-bath. At the indicated times, an aliquot (0.2 ml) was taken up into CH_3CN (1.8 ml). To the mixture was added Celite (10 mg) followed by centrifugation at 2 000 g for 5 min, and then 10 μl of the resultant supernatant was analysed with a Shimadzu LC-5A h.p.l.c. system, on a Zorbax C8 Column (Dupont; 4.6 × 250 mm). For the observation of the formation of the *N*-deacetylated compound (**6a**), the operating conditions of h.p.l.c. were as follows; flow rate, 1.5 ml min^{-1}; carrier, water–MeOH (8:2); monitor at 240 nm. Retention times of the glucosides (**7a**) and (**6a**) were 7.81 and 6.39 min, respectively. In the determination of aniline and azoxybenzene, the carrier used was water–CH_3CN (1:1) containing 0.02% of desferal methanesulphonate) and 1mM-aqueous EDTA. Aniline (at 254 nm) and azoxybenzene (at 305 nm) were monitored spectrophotometrically. When bis-(*p*-nitrophenyl) phosphate was added to the incubation mixture, its DMSO solution was used (final concentration 10^{-3}M).

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