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Note

Synthesis of acetylated methyl (β-D-glucopyranosid)uronates of *N*-aryl-*N*-hydroxyacetamides by the orthoester glycosylation method

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It is generally recognized[1,2] that the carcinogenicity of nitro and amino aromatics derives from metabolic transformation, through reductive and oxidative reactions, respectively, into *N*-hydroxy-*N*-arylamines, and into their *N*-acetylated compounds, namely, *N*-aryl-*N*-hydroxyacetamides (*N*-arylacetohydroxamic acids), although these are not electrophilic *per se* and do not react with nucleophilic sites in critical, cellular macromolecules[3]. Therefore, further biotransformation of these proximate carcinogens into electrophilic species (ultimate carcinogens) has been postulated[4]. *N*-Aryl-*N*-hydroxyacetamide D-glucosiduronic acids are considered[5] to be one of the ultimate carcinogens; however, their chemical and biological characteristics have not been fully investigated.

The first chemical synthesis of acetyl derivatives of these methyl D-glucosiduronates was achieved by Irving[6] and Fishman *et al.*[7], using the Koenigs–Knorr method, although the yields were poor. We now report the synthesis of the acetylated methyl β-D-glucosiduronates of *N*-aryl-*N*-hydroxyacetamide derivatives of chlorophenyl 4-nitrophenyl ethers, widespread herbicides[8] finding use all over the world, including one [4-(2,4-dichlorophenoxy)nitrobenzene] which has been reported to be carcinogenic[9]. The general strategy of this synthesis of the title compounds was based on the orthoester glycosylation method[10], by which D-glucosides of *N*-aryl-*N*-hydroxyacetamides have been synthesized[11].

RESULTS AND DISCUSSION

Treatment of methyl (2,3,4-tri-*O*-acetyl-α-D-glucopyranosyl bromide)uronate (**1**) with the respective *N*-aryl-*N*-hydroxyacetamide (**2a–c**) in the presence of 2,6-lutidine at 70–75° gave the corresponding orthoester (**3a–c**) in good yield (see Scheme 1); the reaction did not proceed at room temperature, even after 4 days. On the other hand, in the synthesis of D-glucopyranosides of **2a–c**, it had been found[11] that the corresponding orthoesters (**4a–c**) are readily formed during 30–42

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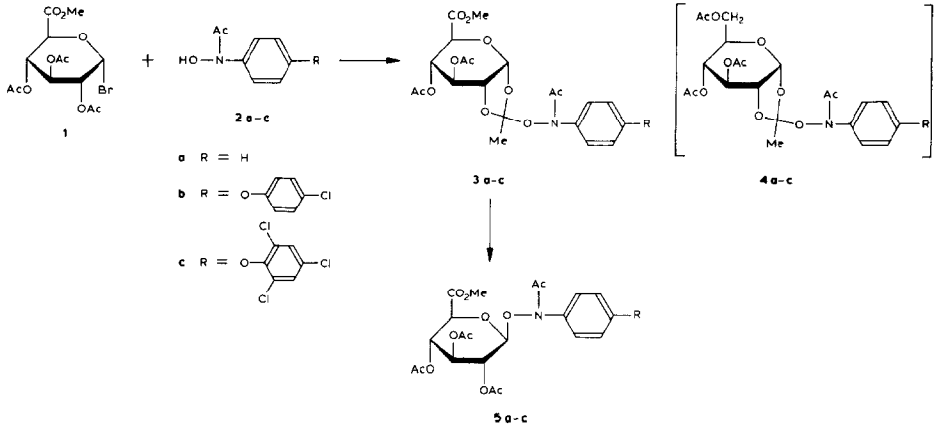

h at 37°. This difference in tendency toward orthoester formation probably derives from the acetoxyl group on C-6 of 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide, which facilitates formation of the orthoesters **4a–c** through a 1,6-acetoxonium intermediate[12]. In the ^{1}H-n.m.r. spectra of compounds **3a–c**, the anomeric protons resonate at δ 5.70–5.72 as doublets (*J* 4.8–4.9 Hz), and the signals of the protons of the methyl group attached to the dioxolane ring occur at δ 1.83–1.84. These facts indicated that *exo*-orthoesters having a *cis*-fused dioxolane ring were formed exclusively[13,14].

Conversion of the orthoesters **3a–c** into **5a–c** was accomplished by refluxing in dichloroethane in the presence of a catalytic amount of 2,6-lutidinium perchlorate[10]. Compounds **5a–c** could be obtained in moderate yield by column chromatography.

Alternative approaches to the synthesis of compounds **5a–c** by the Koenigs–Knorr method[15], one of the most useful methods for 1,2-*trans*-glycosylation, resulted in a complicated mixture, owing to the radical-mediated, degradation reaction[16] of the *N*-aryl-*N*-hydroxyacetamides **2a–c**. Isolated yields of **5a–c** were much lower than those obtained by the orthoester method.

The structures of compounds **5a–c** were confirmed by the i.r. spectral data. The i.r. spectra of compounds **5a–c** exhibited amide I absorption bands at 1690–1685 cm^{-1}, whereas those of **2a–c** were observed at 1620–1610 cm^{-1} owing to the characteristic, intramolecular hydrogen-bonding[17], which indicated that **5a–c** contain an N–O–C-1 linkage. The signals for the anomeric protons of compounds **5a–c** were obscured by overlapping with other proton signals in the ^{1}H-n.m.r. spectra ($CDCl_3$), but assignment of the β configuration to compounds **5a–c** was based on the following considerations: (*i*) H-5 resonated at δ 4.12–3.96, and the signals for H-1, H-2, H-3, and H-4 were observed in a narrow range of ~0.4–0.6 p.p.m., characteristic of β-D-glucopyranosiduronates[18]; (*ii*) compounds **5a–c** prepared by the orthoester method were identical with those obtained by the Koenigs–Knorr method, both of which methods are known to give 1,2-*trans*-glycosides; (*iii*) compounds **5a–c** showed large, negative values of specific rotation; and (*iv*) in the ^{1}H-

n.m.r. spectrum (200 MHz) of **5a** in Me_2DO-d_6, assignment of sugar protons was readily made by homonuclear decoupling experiments, and the J value of the anomeric proton (9.5 Hz) was characteristic[19] for the β configuration of D-gluco-pyranuronates.

In the ^{1}H-n.m.r. spectra of **2a–c** and **5a–c**, the methyl protons assigned to the *N*-acetyl groups resonated at δ 2.17–2.19 (ref. 11), and 2.24–2.34, respectively. The downfield shifts of 0.2–0.3 p.p.m. (compared to those of *N*-arylacetamides) is presumed to be characteristic for *N*-aryl-*N*- hydroxyacetamide derivatives, probably because of the electron-withdrawing inductive effect and/or intra-molecular hydrogen-bonding between the amide carbonyl group and the *N*-hydroxy group in the case of compounds **2a–c**.

The electron-impact, mass spectrum of compound **5a** exhibited no molecular ion, but gave the value *m/z* 317, presumably formed by elimination of the *N*-acetyl-*N*-phenylnitroxyl radical; and subsequent fragmentation-ions at *m/z* 257, 197, 173, 155 (base peak), and 127, were observed. This fragmentation pattern is prominent for the acetylated methyl D-glucopyranosiduronates reported[20]; however, *m/z* 150 (or 151), formed by heterolytic cleavage of the N–O bond, and an M − 42 ion, formed through the elimination of ketene (characteristic peaks for unsubstituted *N*-aryl-*N*-hydroxyacetamides[21]) were not detected.

EXPERIMENTAL

General methods. — Melting points were determined by the capillary method, and are uncorrected. Optical rotations were measured with a Jasco Dip-4 digital polarimeter. T.l.c. was performed on Silica gel $60F_{254}$ (Merck 5554), and column chromatography was conducted on Silica gel (Merck 7734). I.r. and u.v. spectra were respectively recorded with a Jasco A-102 spectrometer and a Shimadzu UV-200S spectrophotometer. ^{1}H-N.m.r. spectra were recorded with a JEOL JNM-FX100 or -FX200 spectrometer for solutions in $CDCl_3$; chemical shifts (δ) are reported with reference to tetramethylsilane, and coupling constants are expressed in Hz. Electron-impact mass spectra were recorded with a Shimadzu-LKB 9000B, using a direct inlet system.

General procedure for the preparation of the orthoesters **3a–c**. — To a solution of methyl (2,3,4-tri-*O*-acetyl-α-D-glucopyranosyl bromide)uronate (**1**; 1.99 g, 5 mmol) and the respective *N*-aryl-*N*-hydroxyacetamide (**2a–c**; 5.1 mmol) in nitro methane (7.5 mL) was added 2,6-lutidine (1.25 mL, 10.8 mmol), and the mixture was heated for 10 h at 70–75°. After cooling, ether (40 mL) was added, and the 2,6-lutidinium bromide was filtered off. The filtrate was diluted with ether (15 mL) and washed successively with water, cold, saturated, aqueous $NaHCO_3$, and water, dried (Na_2SO_4), and evaporated to a brown residue which was chromatographed on Silica gel by elution with 1:3 ethyl acetate–benzene. Physical constants for the orthoesters **3a–c** were as follows.

Methyl 3,4-di-O-acetyl-1,2-O-[1-(N-phenylaminooxy)ethylidene]-α-D-glucopyranuronate (**3a**). — Yield 81%, a colorless syrup; $[\alpha]_D^{21}$ +4.2° (*c* 1.1, ethanol); ^{1}H-n.m.r. (100 MHz; $CDCl_3$): δ 7.42–7.30 (m, 5 H, arom.), 5.72 (d, 1 H, *J* 4.9 Hz, H-1), 5.16–5.08 (m, 2 H), 4.30–4.20 (m, 2 H), 3.75 (s, 3 H, CO_2Me), 2.26 (s, 3 H, NAc), 2.08 (s, 3 H, OAc), 2.06 (s, 3 H, OAc), and 1.84 (s, 3 H, CMe); $\nu_{max}^{CHCl_3}$ 3030–2960 (CH), 1755 and 1220 (ester), and 1690 cm^{-1} (amide); λ_{max}^{EtOH} 240 nm (ε_{mM} 5.90).

Anal. Calc. for $C_{21}H_{25}NO_{11}$: C, 53.96; H, 5.39; N, 3.00. Found: C, 53.74; H, 5.44; N, 3.12.

Methyl 3,4-di-O-acetyl-1,2-O-{1-[N-acetyl-N-4-(4-chlorophenoxy)phenylaminooxy]ethylidene}-α-D-glucopyranuronate (**3b**). — Yield 84%, a colorless syrup; $[\alpha]_D^{21}$ +1.2° (*c* 0.9, ethanol); ^{1}H-n.m.r. (100 MHz; $CDCl_3$): δ 7.32 (d, 2 H, *J* 9.0 Hz), 7.29 (d, 2 H, *J* 9.0 Hz), 6.96 (d, 4 H, *J* 9.0 Hz), 5.70 (d, 1 H, *J* 4.9 Hz, H-1), 5.18–5.09 (m, 2 H), 4.30–4.22 (m, 2 H), 3.76 (s, 3 H, CO_2Me), 2.24 (s, 3 H, NAc), 2.08 (s, 3 H, OAc), 2.05 (s, 3 H, OAc), and 1.83 (s, 3 H, CMe); $\nu_{max}^{CHCl_3}$ 3040–2970 (CH), 1755 (ester), 1685 (amide), and 1240 cm^{-1} (ether); λ_{max}^{EtOH} 249 nm (ε_{mM} 13.20).

Anal. Calc. for $C_{27}H_{28}ClNO_{12}$: C, 54.59; H, 4.75; N, 2.36. Found: C, 54.40; H, 4.88; N, 2.41.

Methyl 3,4-di-O-acetyl-1,2-O-{1-[N-acetyl-N-4-(2,4,6-trichlorophenoxy)-phenylaminooxy]ethylidene}-α-D-glucopyranuronate (**3c**). — Yield 80%, a colorless syrup; $[\alpha]_D^{21}$ +3.4° (*c* 1.0, ethanol); ^{1}H-n.m.r. (100 MHz; $CDCl_3$): δ 7.42 (s, 2 H), 7.27 (d, 2 H, *J* 8.8 Hz), 6.84 (d, 2 H, *J* 8.8 Hz), 5.70 (d, 1 H, *J* 4.8 Hz, H-1), 5.16–5.10 (m, 2 H), 4.28–4.20 (m, 2 H), 3.73 (s, 3 H, CO_2Me), 2.26 (s, 3 H, NAc), 2.08 (s, 3 H, OAc), 2.05 (s, 3 H, OAc), and 1.84 (s, 3 H, CMe); $\nu_{max}^{CHCl_3}$ 3030–2960 (CH), 1755 (ester), 1690 (amide), and 1250 cm^{-1} (ether); λ_{max}^{EtOH} 243 nm (ε_{mM} 14.60) and 287 nm (sh, ε 1800).

Anal. Calc. for $C_{27}H_{26}Cl_3NO_{12}$: C, 48.92; H, 3.95; N, 2.11. Found: C, 48.70; H, 3.86; N, 2.24.

Methyl (acetanilide-N-yl 2,3,4-tri-O-acetyl-β-D-glucopyranosid)uronate (**5a**). — A solution of orthoester **3a** (234 mg, 0.5 mmol) in anhydrous dichloroethane (10 mL) was refluxed in the presence of 2,6-lutidinium perchlorate (2 μmol) for 16 h, cooled, and evaporated, and the residue was chromatographed on a column of silica gel; elution with 1:5 ethyl acetate–benzene gave the starting material (**3a**; 65 mg), and then the corresponding D-glucosiduronate **5a** (94 mg, 40%); m.p. 156–157° (from EtOH–H_2O), $[\alpha]_D^{21}$ −37.5° (*c* 0.9, $CHCl_3$); ^{1}H-n.m.r. (100 MHz; $CDCl_3$): δ 7.45–7.25 (m, 5 H), 5.30–5.14 (m, 3 H), 4.96–4.91 (m, 1 H), 4.06–3.96 (m, 1 H, H-5), 3.78 (s, 3 H, CO_2Me), 2.34 (s, 3 H, NAc), 2.02 (s, 9 H, OAc); (200 MHz; Me_2SO-d_6): δ 7.46–7.30 (m, 5 H), 5.42 (d, 1 H, $J_{1,2}$ 9.5 Hz, H-1), 5.37 (d, 1 H, $J_{3,4}$ 9.5 Hz, H-3), 5.02 (dd, 1 H, $J_{2,3}$ 4.2 Hz, H-2), 5.01 (t, 1 H, H-4), 4.56 (d, 1 H, $J_{4,5}$ 10.0 Hz), 3.63 (s, 3 H, CO_2Me), 2.25 (s, 3 H, NAc), 1.98 (s, 3 H, OAc), 1.96 (s, 3 H, OAc), and 1.94 (s, 3 H, OAc); ν_{max}^{KBr} 2950 (CH), 1750 and 1220 (ester), and 1690 cm^1 (amide); λ_{max}^{EtOH} 240 nm (ε_{mM} 5.20).

Anal. Calc. for $C_{21}H_{25}NO_{11}$: C, 53.96; H, 5.39; N, 3.00. Found: C, 53.79; H, 5.36; N, 2.73.

*Methyl [4'-(4-chlorophenoxy)acetanilide-*N*-yl 2,3,4-tri-*O*-acetyl-β-*D*-glucopyranosid]uronate* (**5b**). — A solution of orthoester **3b** (240 mg, 0.4 mmol) in anhydrous dichloroethane (10 mL) was refluxed in the presence of 2,6-lutidinium perchlorate (2 μmol) for 18 h, cooled, and evaporated, and the residue chromatographed on a column of silica gel; elution with 1:10 acetone–benzene gave the starting material (**3b**; 80 mg), and then the D-glucosiduronate (**5b**; 60 mg, 25%); m.p. 153.5–154.5° (from EtOH–H_2O), $[\alpha]_D^{21}$ −40.0° (*c* 0.8, $CHCl_3$); ^{1}H-n.m.r. (100 MHz; $CDCl_3$): δ 7.33 (d, 2 H, *J* 9.0 Hz), 7.28 (d, 2 H, *J* 9.0 Hz), 6.96 (d, 4 H, *J* 9.0 Hz), 5.35–5.04 (m, 3 H), 4.98–4.88 (m, 1 H), 4.10–3.98 (m, 1 H, H-5), 3.76 (s, 3 H, CO_2Me), 2.30 (s, 3 H, NAc), 2.02 (s, 6 H, OAc), and 2.00 (s, 3 H, OAc); ν_{max}^{KBr} 2970 (CH), 1760 and 1230 (ester), 1690 (amide), and 1245 cm^{-1} (ether): λ_{max}^{EtOH} 247 nm (ε_{mM} 15.40).

Anal. Calc. for $C_{27}H_{28}ClNO_{12}$: C, 54.59; H, 4.75; N, 2.36. Found: C, 54.37; H, 4.73; N, 2.45.

*Methyl [4'-(2,4,6-trichlorophenoxy)acetanilide-*N*-yl 2,3,4-tri-*O*-acetyl-β-*D*-glucopyranosid]uronate* (**5c**). — A solution of orthoester **3c** (264 mg, 0.4 mmol) in anhydrous dichloroethane (8 mL) was refluxed in the presence of 2,6-lutidinium perchlorate (2 μmol) for 18 h, cooled, and evaporated, and the residue chromatographed on a column of silica gel; elution with 1:10 acetone–benzene gave the starting material (**3c**; 85 mg), and then the D-glucosiduronate **5c** (55 mg, 21%); m.p. 180–181° (*i*-PrOH), $[\alpha]_D^{21}$ −48.0° (*c* 0.9, $CHCl_3$); ^{1}H-n.m.r. (100 MHz; $CDCl_3$): δ 7.43 (s, 2 H), 7.27 (d, 2 H, *J* 8.8 Hz), 6.82 (d, 2 H, *J* 8.8 Hz), 5.40–5.00 (m, 3 H), 4.98–4.82 (m, 1 H), 4.12–3.96 (m, 1 H, H-5), 3.75 (s, 3 H, CO_2Me), 2.30 (s, 3 H, NAc), 2.01 (s, 6 H, OAc) and 1.97 (s, 3 H, OAc); ν_{max}^{KBr} 2970 (CH), 1760 and 1220 (ester and ether), and 1680 cm^{-1} (amide); λ_{max}^{EtOH} 240 (ε_{mM} 14.80) and 287 nm (sh, 1.90).

Anal. Calc. for $C_{27}H_{26}Cl_3NO_{12}$: C, 48.92; H, 3.95; N, 2.11. Found: C, 49.12; H, 4.08; N, 2.01.

General procedure for the preparation of **5a–c** *by the Koenigs–Knorr reaction.* — A solution of **1** (1.55 g, 3.9 mmol) and the respective *N*-aryl-*N*-hydroxyacetamide **2a–c** (2 mmol) in benzene (30 mL) was stirred in the presence of silver(I) carbonate (1.36 g, 4.9 mmol) for 24 h in the dark, filtered, and the filtrate evaporated; the residue was chromatographed on a column of silica gel by elution with 1:3 ethyl acetate–benzene. A fraction containing the product was evaporated, and the residue was further purified by preparative t.l.c. with 1:5 ethyl acetate–benzene as the developer to give the D-glucosiduronates **5a, 5b,** and **5c** in 8, 6, and 6% yield, respectively.

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