Migration of the *O*-*p*-Nitrophenyl Group. Mechanism whereby *p*-Nitrophenyl α-D-glycosides Liberate *p*-Nitrophenoxide in Alkaline Solution

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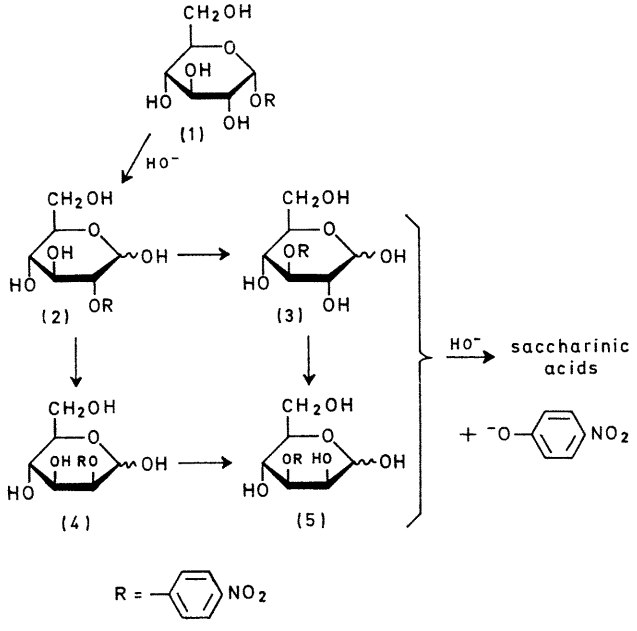
Summary The liberation of *p*-nitrophenoxide from *p*-nitrophenyl-α-D-glucopyranoside (**1**) in alkaline solution involves initial O-1 → O-2 migration of the *p*-nitrophenyl group to give 2-*O*-*p*-nitrophenyl-D-glucose (**2**), followed by subsequent O-2 → O-3 migration to give the 3-ether (**3**) [the D-*manno*-analogues (**4** and **5**) of (**2**) and (**3**) are also formed]; in the final step the 3-ethers are converted into saccharinic acids with the release of *p*-nitrophenoxide anion.

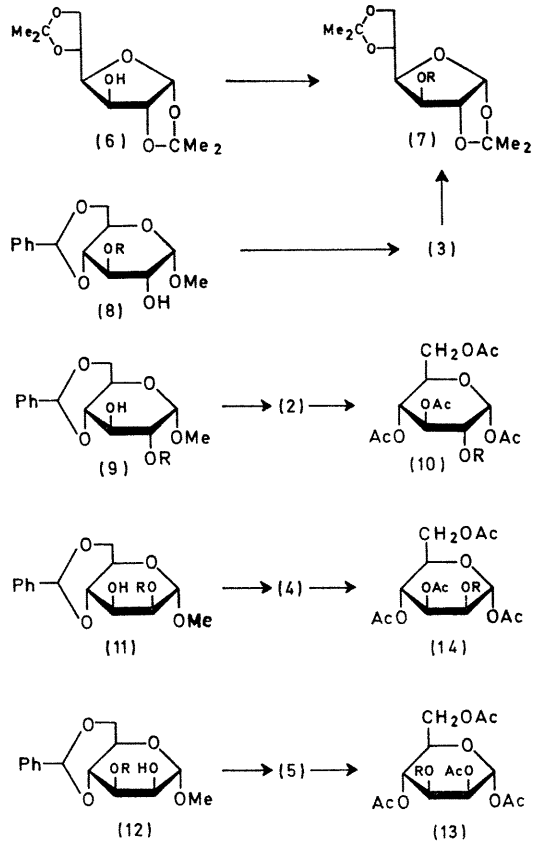
As mixed, full acetals, the glycosides are normally stable towards base, but certain types, such as aryl glycosides, are split by alkali.[1] *p*-Nitrophenyl α-D-glucopyranoside (**1**) very readily liberates *p*-nitrophenoxide in aqueous alkali;[2] the release is about 10^5 times faster[3] than the release of phenoxide from phenyl α-D-glucopyranoside in 3·9M-potassium hydroxide at 60°. Although *p*-nitrophenyl glycosides are frequently used as model substrates for studying the action of glycosidase enzymes, no evidence has been presented to explain the exceptionally fast hydrolysis of (**1**) in alkaline media. It has been speculated[4,5] that the *p*-nitrophenoxide anion is released from (**1**) by nucleophilic attack of hydroxide ion, either at C-1 (with glucosyl–oxygen fission) or at the aryl ring (with aryl–oxygen fission).

Data here presented show that the release of *p*-nitrophenoxide from (**1**) in alkali proceeds by a three-stage process not hitherto considered, and involves a base-catalysed O → O migration of the *p*-nitrophenyl group, presumably *via* an intramolecular, nucleophilic, aromatic substitution-reaction. The primary product, 2-*O*-*p*-nitrophenyl-D-glucose (**2**), has been isolated and characterized. Products subsequently formed include 3-*O*-*p*-nitrophenyl-D-glucose (**3**), which is presumably formed from (**2**) by O-2 → O-3 migration of the aryl group, together with the 2-epimers (**4** and **5**) of the ethers (**2**) and (**3**), respectively. The structures of (**2**), (**3**), (**4**), and (**5**) are supported by independent synthesis. In the step when *p*-nitrophenoxide is released, presumably from the 3-ethers (**3** and **5**), the sugar residue is converted into a saccharinic acid[5] and no D-glucose is liberated.



Thus, treatment of (**1**) (1·0 g) in aqueous 0·25M-potassium hydroxide (35 ml) for 1·75 h at 25° caused a very small release of *p*-nitrophenoxide but almost complete conversion of (**1**) into a mixture (isolated yield 87%) of four new products. These were partially resolved by careful column chromatography to yield (**2**) (R_F 0·38), (**3**) (R_F 0·41), (**4**) (R_F 0·37), and (**5**) (R_F 0·36); the last two were incompletely separated (t.l.c., silica gel, 20 : 3 ethyl acetate–methanol). All four products reacted as reducing sugars with aniline hydrogen phthalate and contained a *p*-nitrophenyl residue (i.r. and n.m.r. spectroscopy). With the sulphuric acid spray-reagent, the 2-ethers (**2** and **4**) gave brown spots and the 3-ethers (**3** and **5**) black spots. Heating a solution of (**2**), (**3**), (**4**), and (**5**) in 0·05M-potassium hydroxide at 80° led within 15 min to complete release of *p*-nitrophenyl groups as *p*-nitrophenoxide and formation of a non-reducing product(s) having R_F 0·32 (4 : 1 : 1 butanol–acetic acid–water), which was detected with a permanganate–periodate spray-reagent. Acidification (Amberlite IR-120, H^+, resin) of the solution gave a new product (R_F 0·53) showing λ_{max} (film) 2·98 and 5·70 μm; these data are identical with the properties of glucometasaccharinic acids prepared from laminaran.[6]

The critical role of the 2-hydroxy-group in the alkaline degradation of (**1**) was verified by the observation that, at pH 10·25 and 85°, *p*-nitrophenyl-2,3-di-*O*-methyl-α-D-glucopyranoside liberates *p*-nitrophenoxide 7 × 10^5 times more slowly than (**1**), whereas *p*-nitrophenyl-3-*O*-methyl-α-D-glucopyranoside reacts 1·45 times faster than (**1**). In 0·25M-potassium hydroxide at 25° the 2,3-diether is stable, but the 3-ether is rapidly converted into a reducing-sugar derivative containing the aryl group.



The structures of (**2**), (**3**), (**4**), and (**5**) were established by chemical transformations, n.m.r. spectroscopy of their tetra-acetates, and synthesis. Hot methanolic hydrogen chloride converted (**2**) into a glycoside that was decomposed by periodate (indicating that it was not a 3-ether), whereas similar treatment of (**3**) gave a periodate-resistant glycoside, indicating substitution at C-3. Treatment of (**3**) with acetone in the presence of copper(II) sulphate and toluene-*p*-sulphonic acid gave 1,2 : 5,6-di-*O*-isopropylidene-3-*O*-*p*-nitrophenyl-α-D-glucopyranose (**7**), m.p. 131—132°, $[\alpha]_D^{23}$ −43·2° (*c* 1, chloroform), identical with the product obtained by treatment of 1,2 : 5,6-di-*O*-isopropylidene-α-D-glucofuranose (**6**) with 1-fluoro-4-nitrobenzene in the presence of potassium hydroxide.

Treatment of methyl 4,6-*O*-benzylidene-α-D-glucopyranoside (3·0 g) with 1-fluoro-4-nitrobenzene (0·9 moles) and potassium hydroxide in dimethyl sulphoxide, with subsequent column chromatography, gave 32% of methyl 4,6-*O*-benzylidene-3-*O*-*p*-nitrophenyl-α-D-glucopyranoside (**8**), m.p. 159—160°, $[\alpha]_D^{23}$ +223° (*c* 1, chloroform), black

spot on t.l.c. (H_2SO_4), and 10% of methyl 4,6-*O*-benzylidene-2-*O*-*p*-nitrophenyl-α-D-glucopyranoside (**9**), m.p. 184—185°, $[\alpha]_D^{23}$ +18° (*c* 1, chloroform), brown spot on t.l.c. (H_2SO_4). Debenzylidenation of (**8**) with trifluoroacetic acid–water, followed by hydrolysis of the glycoside with 3M-sulphuric acid gave (**3**). Similar treatment of (**9**) gave (**2**) which, upon acetylation with acetic anhydride–zinc chloride gave 1,3,4,6-tetra-*O*-acetyl-2-*O*-*p*-nitrophenyl-α-D-glucopyranose (**10**), m.p. 168—169°, $[\alpha]_D^{23}$ +16.8° (*c* 1, chloroform), identical with the product obtained by similar acetylation of (**2**) prepared by treatment of (**1**) with alkali.

By following the conditions used for preparing (**8**) and (**9**), methyl 4,6-*O*-benzylidene-α-D-mannopyranoside gave 16% of methyl 4,6-*O*-benzylidene-2-*O*-*p*-nitrophenyl-α-D-mannopyranoside (**11**) [m.p. 153—154°, $[\alpha]_D^{23}$ −13·1° (*c* 1, chloroform), brown spot on t.l.c.], and 24% of methyl 4,6-*O*-benzylidene-3-*O*-*p*-nitrophenyl-α-D-mannopyranoside (**12**) [m.p 142—143°, $[\alpha]_D^{23}$ +135·3° (*c* 1, chloroform), black spot on t.l.c.]. Removal of the *O*-benzylidene group from (**11**) and acid hydrolysis of the glycoside gave (**4**), and similar treatment of (**12**) gave (**5**). The position of the ether group in (**4**) and (**5**) was established, as for the D-*gluco*-analogues, by treatment of the methyl glycosides with periodate. Acetylation of (**4**) with acetic anhydride–zinc chloride gave syrupy 1,3,4,6-tetra-*O*-acetyl-2-*O*-*p*-nitrophenyl-α-D-mannopyranose (**14**), $[\alpha]_D^{23}$ −22° (*c* 1, chloroform), brown spot on t.l.c., τ ($CDCl_3$) (60 MHz) 3·75 (doublet, $J_{1,2}$ 2 Hz, 1-H), 4·58 (3-H), and 5·10 (2-H). Similar acetylation of (**5**) gave syrupy 1,2,4,6-tetra-*O*-acetyl-3-*O*-*p*-nitrophenyl-α-D-mannopyranose (**13**), $[\alpha]_D^{23}$ +56° (*c* 1, chloroform), black spot on t.l.c., τ ($CDCl_3$) 3·82 (doublet, $J_{1,2}$ 2 Hz, 1-H), 4·68 (2-H), and 5·12 (3-H). Acetylation of the mixture of (**4**) and (**5**) obtained by treatment of (**1**) with alkali gave a mixture of the acetates (**13**) and (**14**) showing, as the only signals in the region for anomeric protons, narrow doublets (*J* 2 Hz) at τ 3·75 and 3·82.

In alkaline solution the β-analogue of (**1**) also exhibits migration of the *p*-nitrophenyl group, but at a lower rate.

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