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Note

Carbonate derivatives of methyl D-glucopyranosides

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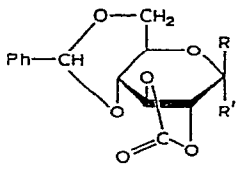
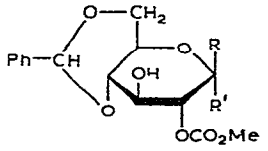
The five-membered cyclic carbonate ring involving HO-2 and HO-3 of α-D-glucopyranose structures[1,2] is susceptible to nucleophilic attack, whereupon ring-opening occurs[2]. For example, methanol gives 2-*O*- and 3-*O*-methoxycarbonyl derivatives. Since both cyclic and acyclic carbonate groups are susceptible to nucleophilic attack, it seemed possible that these derivatives might function as active-site-directed, irreversible inhibitors of enzymes for which D-glucose and D-glucose derivatives are the substrates.

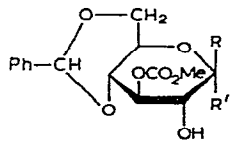
Doane *et al.*[1] used ethyl chloroformate to introduce the 2,3-cyclic carbonate group into methyl 4,6-*O*-benzylidene-α-D-glucopyranoside. Treatment of the product (**1a**) with methanol–triethylamine gave a mixture of the 2-*O*-(**2a**) and 3-*O*-methoxycarbonyl (**3a**) derivatives which were separated by preparative t.l.c.[2]. We have used essentially similar methods to prepare the corresponding compounds (**2b** and **3b**) in the β-series from **1b**. The 4,6-*O*-benzylidene groups were conveniently removed from **1b**, **2b**, and **3b** by catalytic hydrogenation to give methyl β-D-glucopyranoside 2,3-



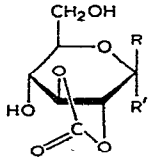
1a R = H, R′ = OMe
b R = OMe, R′ = H



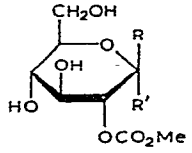
2a R = H, R′ = OMe
b R = OMe, R′ = H



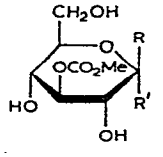
3a R = H, R′ = OMe
b R = OMe, R′ = H



4a R = H, R′ = OMe
b R = OMe, R′ = H



5a R = H, R′ = OMe
b R = OMe, R′ = H



6a R = H, R′ = OMe
b R = OMe, R′ = H

carbonate (**4b**), and methyl 2-*O*-(**5b**) and 3-*O*-methoxycarbonyl-α-D-glucopyranoside (**6b**). The corresponding compounds in the α-series (**4a**, **5a**, and **6a**) were prepared analogously.

In the α-series, Doane *et al.*[2] assigned the position of the methoxycarbonyl groups on the basis of the strong deshielding effect by the carbonyl moiety on the proton attached to the same ring carbon. The low-field, ring-proton signals in **2a** and **3a** were readily identified from their splitting patterns (H-2 in **2a**, $J_{1,2}$ 3.8, $J_{2,3}$ 9.8 Hz; H-3 in **3a**, $J_{2,3} = J_{3,4} = 9.5$ Hz).

In the β-series, H-2 in **2b** and H-3 in **3b** are in *trans*-diaxial relationship with the vicinal protons, and the signals appear as triplets (H-2 in **2b**, $J_{1,2} = J_{2,3} = 8.5$ Hz; H-3 in **3b**, $J_{2,3} = J_{3,4} = 9.2$ Hz. These assignements are confirmed by a comparison of the chemical shifts for H-2 and H-3 in the β-series (H-2 in **2b**, τ 5.30; H-3 in **3b**, τ 5.00) with those (H-2 in **2a**, τ 5.35; H-3 in **3a**, τ 4.90) established[2] for the α-D anomers.

The 2-*O*-(**5a,b**) and 3-*O*-methoxycarbonyl-(**6a,b**), and 2,3-cyclic carbonate derivatives (**4a,b**) of methyl α- and β-D-glucopyranoside were then tested as inhibitors of dextranase, yeast α-D-glucosidase, emulsin (β-D-glucosidase), and *N*-acetyl-β-D-glucosaminidase. Neither of the cyclic carbonates had any effect on any of the enzymes, and none of the remaining compounds had any effect on dextranase.

The β-compounds **5b** and **6b** caused small losses of activity of the β-D-glucosidase (9 and 4%) and *N*-acetyl-β-D-glucosaminidase (10 and 3%) at pH 5.0, which is near their pH optima; at pH 6.8, no loss of activity was caused. Compounds in the α-series had no effect on these enzymes at any pH.

The most-striking result was obtained with yeast α-D-glucosidase, since **5a** and **6a** each caused significant losses of activity (89 and 61%), respectively). The data in Table I also indicate that the 2-*O*-methoxycarbonyl derivative (**5a**) is a more-effective inhibitor than the 3-*O*-methoxycarbonyl compound (**6a**). Evidence that the loss of activity is caused by interaction at the active site is afforded by the observations

TABLE I

EFFECT OF ERYTHRITOL ON THE INACTIVATION OF YEAST α-D-GLUCOSIDASE BY THE CARBONATE DERIVATIVES **5a** AND **6a**

Treatment	*Inactivation* (%)		
	Time of treatment (*h*)		
	0	*2*	*4*
5a	54	81	89
5a plus erythritol (3.9×10^{-1} M)	30	41	63
6a	40	52	61
6a plus erythritol (3.9×10^{-1} M)	21	41	58

(Table I) that the enzyme was protected by erythritol (a good competitive inhibitor of α-D-glucosidase).

Although we regard these results as preliminary, they suggest that the inhibition of yeast α-D-glucosidase by methyl 2-*O*- and 3-*O*-methoxycarbonyl-α-D-glucopyranoside is selective, progressive, and subject to inhibitor protection, and indicate that carbonates of this type may be valuable as active-site-directed, irreversible, enzyme inhibitors for glycosidases. Work is in progress to establish the nature of the inhibition process.

EXPERIMENTAL

Materials and methods. — Dextranase (EC 3.2.1.11) and β-D-glucosidase (emulsin, EC 3.2.1.2) were obtained from Koch–Light Laboratories Ltd., and yeast α-D-glucosidase (EC 3.2.1.20) from Sigma Chemical Co. Ltd. 2-Acetamido-2-deoxy-β-D-glucoside acetamidodeoxyglucohydrolase (*N*-acetyl-β-D-glucosaminidase, EC 3.2.1.30) was obtained[3] from fresh hen-egg white.

T.l.c. was performed on silica gel G (Merck, 7731), using *A* ethyl acetate, *B* ethyl acetate–hexane (1:1), *C* ether, *D* benzene–ether (1:1), *E* methanol–ether (1:5), *F* chloroform–acetone* (3:1), and detection with iodine vapour or vanillin–sulphuric acid[4]. Methanol[5] and triethylamine[6] were purified before use.

β-D-Glucosidase activity was determined at pH 5.0, using *o*-nitrophenyl β-D-glucopyranoside[7]. Dextranase was assayed against dextran at pH 5.0, the reducing sugar produced being determined by the 3,5-dinitrosalicylic acid method[8]. *N*-Acetyl-β-D-glucosaminidase was assayed[9] at pH 4.0 against *p*-nitrophenyl 2-acetamido-2-deoxy-β-D-glucopyranoside. α-D-Glucosidase was assayed at pH 6.8 against *p*-nitrophenyl α-D-glucopyranoside.

Methyl 2-O-methoxycarbonyl-α-D-glucopyranoside (**5a**). — A solution of methyl 4,6-*O*-benzylidene-2-*O*-methoxycarbonyl-α-D-glucopyranoside[10] (**2a**, 200 mg) in dry methanol (30 ml) was hydrogenated in the presence of palladium catalyst (10% on charcoal, 150 mg) for 20 h. The mixture was then filtered and concentrated, and the residue was dried *in vacuo* over phosphorus pentaoxide to give **5a** as a hygroscopic solid (145 mg, 98%), $[\alpha]_D^{20}$ +127° (*c* 1, chloroform), single vanillin-positive spot (R_F 0.3, solvent *A*), ν_{max}^{Nujol} 1750 cm^{-1} (C=O, acyclic carbonate); lit.[10] $[\alpha]_D^{20}$ +127.7° (*c* 1, chloroform).

Methyl 3-O-methoxycarbonyl-α-D-glucopyranoside (**6a**). — Hydrogenation of methyl 4,6-*O*-benzylidene-3-*O*-methoxycarbonyl-α-D-glucopyranoside[10] (**3a**, 300 mg), as described above, gave **6a** as a hygroscopic solid (200 mg, 90.1%), $[\alpha]_D^{20}$ +158° (*c* 1, methanol), single vanillin-positive spot (R_F 0.3, solvent *A*), ν_{max}^{Nujol} 1750 cm^{-1} (C=O, acyclic carbonate) (Found: C, 42.7; H, 6.3. $C_9H_{16}O_8$ calc.: C, 42.9; H, 6.3%).

Methyl α-D-glucopyranoside 2,3-carbonate (**4a**). — Hydrogenation of methyl 4,6-*O*-benzylidene-α-D-glucopyranoside 2,3-carbonate[10] (**1a**, 500 mg), as described

*Chloroform–acetone mixtures can be extremely hazardous, especially on storage and in the presence of base [H. K. King, *Chem. Britain*, 6 (1970) 231].

above, gave **4a** (315 mg, 90%), $[\alpha]_D^{20}$ +128° (*c* 1.6, methanol), single iodine-positive spot (R_F 0.53, solvent *A*), ν_{max}^{Nujol} 1810 cm^{-1} (C=O, cyclic carbonate) (Found: C, 43.9; H, 5.6. $C_8H_{12}O_7$ calc.: C, 43.6; H, 5.5%).

Methyl 4,6-O-benzylidene-β-D-glucopyranoside 2,3-carbonate (**1b**). — A solution of methyl 4,6-*O*-benzylidene-β-D-glucopyranoside[11] (2 g) in *p*-dioxane (60 ml) was treated with ethyl chloroformate (20 ml) and cooled to ~0°. To the stirred solution, a solution of triethylamine (20 ml) in benzene (100 ml) was added dropwise. After 1 h at 5°, the reaction mixture was washed successively with dilute hydrochloric acid and then water until neutral, dried ($MgSO_4$), and concentrated to give **1b** (1.8 g, 82.6%), m.p. 185–186° (from chloroform–ether), $[\alpha]_D^{20}$ −107° (*c* 1, chloroform), single iodine-positive spot (R_F 0.78, solvent *D*), ν_{max}^{Nujol} 1810 and 1840 (C=O, cyclic carbonate) and 700 cm^{-1} (phenyl) (Found: C, 58.2; H, 5.0. $C_{15}H_{16}O_7$ calc.: C, 58.4; H, 5.2%).

Reaction of methyl 4,6-O-benzylidene-β-D-glucopyranoside 2,3-carbonate (**1b**) *with methanol.* — To a solution of **1b** (0.3 g) in chloroform (7.0 ml), dry methanol (0.50 ml) and purified triethylamine (0.20 ml) were added. The solution was kept at room temperature for 40 min and then concentrated under reduced pressure. The crude product (0.32 g) contained two iodine-positive spots having R_F values less than that of the starting material, but no starting material. The mixture (200 mg) was eluted from a column of silica gel (Merck, 7734) with chloroform–acetone mixtures.

The faster-moving component was methyl 4,6-*O*-benzylidene-2-*O*-methoxycarbonyl-β-D-glucopyranoside (**2b**; 83 mg, 41.5%), R_F 0.57 (solvent *D*); when recrystallized from ether–hexane, **2b** had m.p. 92–93°, $[\alpha]_D^{20}$ −72° (*c* 1, chloroform), ν_{max}^{Nujol} 1750 (C=O, acyclic carbonate) and 700 cm^{-1} (phenyl) (Found: C, 56.4; H, 5.9. $C_{16}H_{20}O_8$ calc.: C, 56.5; H, 5.9%).

N.m.r. data ($CDCl_3$): τ 5.65 (d, 1 H, $J_{1,2}$ 8.5 Hz, H-1), 5.3 (t, 1 H, $J_{1,2} = J_{2,3} =$ 8.5 Hz, H-2), 5.7–6.5 (m, H-3,4,5,6), 6.5 (s, 3 H, MeO-1), 6.2 (s, 3 H, COOMe), 4.5 (s, 1 H, PhC*H*), 2.55 (m, 5 H, Ph).

The slower-moving component was methyl 4,6-*O*-benzylidene-3-*O*-methoxycarbonyl-β-D-glucopyranoside (**3b**; 116 mg, 58%), R_F 0.48 (solvent *D*); when recrystallized from ether–hexane, **3b** had m.p. 133–134°, $[\alpha]_D^{20}$ −57° (*c* 1, chloroform), ν_{max}^{Nujol} 1750 (C=O, acyclic carbonate) and 700 cm^{-1} (phenyl) (Found: C, 56.5; H, 5.7%).

N.m.r. data ($CDCl_3$): τ 5.65 (d, 1 H, $J_{1,2}$ 7.3 Hz, H-1), 6.0–6.5 (m, H-2,4,5,6), 5.0 (t, 1 H, $J_{2,3} = J_{3,4} = 9.2$ Hz, H-3), 6.4 (s, 3 H, MeO-1), 6.2 (s, 3 H, COOMe), 4.5 (s, 1 H, PhC*H*), 2.55 (m, 5 H, Ph).

Methyl 2-O-methoxycarbonyl-β-D-glucopyranoside (**5b**). — Hydrogenation of methyl 4,6-*O*-benzylidene-2-*O*-methoxycarbonyl-β-D-glucopyranoside (**2b**, 100 mg), as described above, gave **5b** as a hygroscopic solid (51 mg, 66.2%), $[\alpha]_D^{20}$ −45° (*c* 1, chloroform), single vanillin-positive spot (R_F 0.44, solvent *E*), ν_{max}^{Nujol} 1750 (C=O, acyclic carbonate) (Found: C, 42.9; H, 6.2. $C_9H_{16}O_8$ calc.: C, 42.9; H, 6.3%).

Methyl 3-O-methoxycarbonyl-β-D-glucopyranoside (**6b**). — Hydrogenation of methyl 4,6-*O*-benzylidene-3-*O*-methoxycarbonyl-β-D-glucopyranoside (**3b**, 100 mg),

as described above, gave **6b** as a hygroscopic solid (61 mg, 79.2%), $[\alpha]_D^{20}$ −31° (*c* 1, chloroform), single vanillin-positive spot (R_F 0.43, solvent *E*), ν_{max}^{Nujol} 1750 (C=O, acyclic carbonate) (Found: C, 42.6; H, 6.6. $C_9H_{16}O_8$ calc.: C, 42.9; H, 6.3%).

Methyl β-D-glucopyranoside 2,3-carbonate (**4b**). — Hydrogenation of methyl 4,6-*O*-benzylidene-β-D-glucopyranoside 2,3-carbonate (**1b**, 200 mg), as described above, but in solution in ethyl acetate, gave **4b** (120 mg, 92.3%), m.p. 172–173° (from ethyl acetate–hexane), $[\alpha]_D^{20}$ −54° (*c* 1, methanol), single iodine-positive spot (R_F 0.2, solvent *C*), ν_{max}^{Nujol} 1800 cm^{-1} (C=O, cyclic carbonate) (Found: C, 43.9; H, 5.6. $C_8H_{12}O_7$ calc.: C, 43.6; H, 5.5%).

Enzyme inhibition studies. — Dextranase solution (1 mg/ml in 0.2M citrate buffer, pH 5.0; 0.2 ml) was incubated with a solution of methyl 2-*O*-methoxycarbonyl-α-D-glucopyranoside (4 mg/ml) in methanol (0.1 ml) at 18°. Samples (0.02 ml) were taken at suitable intervals (0, 2, and 4 h) and assayed for dextranase activity, using an appropriate control incubation. The test was also carried out at pH 6.8.

The effect of methyl 3-*O*-methoxycarbonyl-α-D-glucopyranoside, methyl α- and β-D-glucopyranoside 2,3-carbonate, methyl 2-*O*- and 3-*O*-methoxycarbonyl-β-D-glucopyranoside severally on dextranase was also examined.

Using procedures essentially similar to that described above, the effect of each of the foregoing six carbonate derivatives was also examined on *N*-acetyl-β-D-glucosaminidase at its optimum pH (5.0) and at pH 6.8, on β-D-glucosidase at its optimum pH (5.0) and a pH 6.8, and on α-D-glucosidase at its optimum pH (6.8; no tests could be carried out at pH 5.0 because the enzyme then lost activity rapidly).

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