Carbohydrate Research
Elsevier Publishing Company, Amsterdam
Printed in Belgium

Note

Synthesis of substituted phenyl α-D-mannopyranosides

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(Received July 14th, 1969)

Nitrophenyl α-D-mannosides are useful chromogenic substrates for the quantitative determination of α-D-mannosidase activity, and 4-methylumbelliferyl α-D-mannoside and 6-bromo-2-naphthyl α-D-mannoside can be used for histochemical investigations, and for the localisation of α-D-mannosidase activity after analytical, disc electrophoresis on polyacrylamide and electrophoresis on agar gel. In the first case, the enzyme activity is detected by the u.v.-fluorescence of free 4-methylumbelliferone. In the second case; the polyacrylamide gel is incubated for 30 min in a solution of the bromonaphthyl mannoside and then treated with a solution (1 mg/ml) of diazotised 4,4′-bi-*o*-anisidine (4,4′-diamino-3,3′-dimethoxybiphenyl) (Fast Blue B, Fluka) in Tris buffer (pH 8.8). α-D-Mannosidase zones appear red–violet. The first method is the most specific and highly sensitive.

We have now prepared a series of substituted phenyl α-D-mannosides, by using the Helferich[1-3] method, for use in an investigation of the enzymic hydrolysis of these compounds. Fusion of α-D-mannose pentaacetate with the appropriate phenol in the presence of zinc chloride gave the compounds shown in Table I; the deacetylated derivatives are listed in Table II.

EXPERIMENTAL

The purity of the products was tested by t.l.c. on Silica Gel G (Merck) with ethyl acetate–benzene (3:7, v/v) for the mannoside acetates, and acetic acid–water–ethyl acetate (1:1:3, v/v) for the mannosides; detection was with 5% sulphuric acid in ethanol (10 min at 120°). Melting points were determined on a Mettler FP-2 instrument and are uncorrected. Optical rotations were measured with a Perkin–Elmer Model 141 polarimeter.

Preparation of glycosides. — α-D-Mannose pentaacetate[4] (0.05 mole) and the appropriate phenol (0.15–0.20 mole) were fused together and then treated with a solution of freshly fused zinc chloride (6 g) in a mixture (50 ml) of acetic acid–acetic anhydride (95:5, v/v). The melt was heated under diminished pressure for 1 h at 125–135°. The cooled mixture was dissolved in chloroform (500 ml) and washed successively with very dilute hydrochloric acid, ice-cold N sodium hydroxide, and water. The chloroform layer was dried (Na_2SO_4), treated with charcoal, and evapo-

TABLE I

ACETYLATED α-D-MANNOPYRANOSIDES

Aglycon group	*M.p.* (*degrees*)	*Yield* (%)	$[\alpha]_D^{22}$ (*degrees*) (c 2, *chloroform*)	*Found*(%)		*Formula*	*Calc.*(%)	
				C	*H*		*C*	*H*
p-Chlorophenyl	131–132	44	+77.5	52.3	5.4	$C_{20}H_{23}ClO_{10}$	52.3	5.1
p-Cresyl	80–81	53	+69.4	57.3	6.1	$C_{21}H_{26}O_{10}$	57.5	6.0
p-Methoxyphenyl	101–102	35	+95.9	55.6	5.7	$C_{21}H_{26}O_{11}$	55.5	5.4
p-Ethoxyphenyl	73–74	37	+66.4	56.4	5.9	$C_{22}H_{28}O_{11}$	56.4	6.0
p-Bromophenyl	132–133	33	+74.0	47.8	4.5	$C_{20}H_{23}BrO_{11}$	47.7	4.6
p-Ethylphenyl	103–104	48	+70.3	59.0	7.0	$C_{22}H_{28}O_{10}$	59.1	7.0
p-*tert*-Butylphenyl	111–113	28	+93.8	59.7	6.7	$C_{24}H_{30}O_{10}$	60.0	6.7
o-Nitrophenyl	131–133	26	+121.7	51.2	5.0	$C_{20}H_{23}NO_{12}$	51.2	4.9
m-Nitrophenyl	90–92	27	+81.2	51.3	4.9	$C_{20}H_{23}NO_{12}$	51.2	4.9
m-Cresyl	96–97	35	+72.7	57.5	6.0	$C_{21}H_{26}O_{10}$	57.5	6.0
m-Bromophenyl	95–96	35	+77.0	47.8	4.8	$C_{20}H_{23}BrO_{11}$	47.7	4.6
4-Methylumbelliferyl	160–161	37	+136.0	56.4	5.1	$C_{24}H_{27}O_{12}$	56.9	5.1
6-Bromo-2-naphthyl	169–170	45	—	52.0	4.6	$C_{24}H_{25}BrO_{10}$	52.1	4.7

TABLE II

α-D-MANNOPYRANOSIDES

Aglycon group	*M.p.* (*degrees*)	*Crystallisation solvent*	*Yield* (%)	$[\alpha]_D^{22}$ (*degrees*) (c 2, *methanol*)	*Found*(%)		*Formula*	*Calc.*(%)	
					C	*H*		*C*	*H*
p-Chlorophenyl	199–201	water	85	+127.5	49.6	5.4	$C_{12}H_{15}ClO_6$	49.6	5.2
p-Cresyl	166–167	water	88	+122.9	57.5	6.7	$C_{13}H_{18}O_6$	57.7	6.7
p-Methoxyphenyl	155–156	methanol	83	+122.6	54.3	6.3	$C_{13}H_{18}O_7$	54.5	6.3
p-Ethoxyphenyl	162–163	methanol	82	+107.0	55.7	6.6	$C_{14}H_{20}O_7$	56.0	6.7
p-Bromophenyl	207–209	water	84	+117.9[a]	43.7	4.6	$C_{12}H_{15}BrO_6$	43.6	4.5
p-Ethylphenyl	123–125	methanol	85	+106.3	59.0	7.0	$C_{14}H_{20}O_6$	59.1	7.0
p-*tert*-Butylphenyl	—	—	74	+110.7	60.4	7.7	$C_{16}H_{24}O_6$	60.4	7.7
o-Nitrophenyl	183–184	methanol	72	+99.8[b]	47.8	5.1	$C_{12}H_{15}NO_8$	47.8	5.0
m-Nitrophenyl	150–151	methanol	83	+130.8	47.7	5.1	$C_{12}H_{15}NO_8$	47.8	5.0
m-Cresyl	69–70	water	82	+115.9	57.5	6.7	$C_{13}H_{18}O_6$	57.8	6.7
m-Bromophenyl	133–134	water	88	+106.3	43.6	4.6	$C_{12}H_{15}BrO_6$	43.6	4.5
4-Methylumbelliferyl	222–225	water-*p*-dioxane	81	+178.2[a]	56.6	5.4	$C_{16}H_{19}O_8$	56.8	5.3
6-Bromo-2-naphthyl	246–248	dioxan	86	+144.0	49.7	4.3	$C_{16}H_{27}BrO_6$	49.8	4.4

[a] *c* 0.5; [b] *c* 1.0.

rated *in vacuo*. When dark-coloured, condensation products could not be removed with charcoal, solutions of the acetate in benzene were transferred to a column of alumina that was then eluted with more benzene; carbohydrate material was detected by the Molisch test. The products were crystallised from ethanol to constant m.p. and optical rotation (Table I).

The tetra-*O*-acetyl-α-D-mannopyranosides were catalytically deacetylated[5] by using barium methoxide for the nitrophenyl derivatives, and sodium methoxide for the other derivatives. The characteristics of the aryl α-D-mannopyranosides are shown in Table II. The *p*-*tert*-butylphenyl derivative, which has an amorphous structure and no defined m.p., dissolves immediately in all common solvents.

ACKNOWLEDGMENTS

We thank Professor Dr. L. Massart for his interest in this work, and Miss J. De Lat for performing the microanalyses.

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