ISOLATION AND IDENTIFICATION OF AN *O*-(2-HYDROXYPROPYL)-MALTOSE FROM THE FAECES OF RATS FED WITH *O*-(HYDROXYPROPYL)STARCH

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ABSTRACT

When rats were fed with diets containing hydroxypropylated potato-starches with a degree of substitution up to 0.11, the major metabolite, isolated from the faeces, was shown by mass spectrometry and p.m.r. spectrometry of its peracetate to be 4-*O*-{2-*O*-[(*RS*)-2-hydroxypropyl]-α-D-glucopyranosyl}-D-glucopyranose.

INTRODUCTION

O-(Hydroxypropyl)starch, prepared by treating starch with propylene oxide in an alkaline solution[1], is used in the food industry as a thickener, binder, stabilizer, and film-forming agent. In a study of the metabolic fate of *O*-(hydroxypropyl-2-^{14}C)-starch in the rat[2], over 95% of the labelled metabolites were found in the faeces. We now report the identification of the major metabolite as 4-*O*-{2-*O*-[(*RS*)-2-hydroxypropyl]-α-D-glucopyranosyl}-D-glucopyranose.

EXPERIMENTAL

General methods. — Solutions were concentrated under reduced pressure at temperatures below 50° in a rotary film evaporator. Thin-layer chromatography (t.l.c.) was performed on DC-Fertigplatten Kieselgel (Merck), without activation, using sulphuric acid for detection; ethyl acetate–acetic acid–water (5:3:2) was used for oligosaccharides and methanol–benzene (3:97) for acetylated compounds. Column chromatography of the acetylated compounds was effected on silica gel (Merck, 7754). Columns (i.d. 18 mm) were packed with a slurry of adsorbent (25 g) and methanol–benzene (75 ml, 1.5:98.5). Methanol–benzene (0.75:99.25) was used as the eluent; the separations were monitored by t.l.c.

Mass spectra were recorded at 205° and 70 eV with a Varian MAT 731 double-

focussing, mass spectrometer. The exact masses of the ion fragments were determined by peak matching or with a Varian MAT Spectro System™ 100 MS, on-line with the mass spectrometer.

P.m.r. spectra were recorded at 100 MHz and 70° with a Varian HA-100 spectrometer, using tetramethylsilane as the internal standard and solutions in chloroform-*d* at 70–90 mg/ml.

Isolation of the major metabolite. — Combined samples of rat faeces (260 g, collected during a feeding trial in which albino rats of a Wistar strain were fed with diets containing 20–30% of hydroxypropylated potato-starches[1] with degrees of substitution varying from 0.05–0.11) were homogenized in ethanol (750 ml). The suspension was centrifuged, the supernatant was treated with activated carbon (1 g) and centrifuged again, and the clear, yellow supernatant was concentrated. The residue was dissolved in ethanol (50 ml) and an extra amount (400 ml) of ethanol was added with vigorous shaking. After 2 h at room temperature, the suspension was centrifuged and the ethanol layer was evaporated to a syrup (29 g). T.l.c. showed that the syrup contained the same compounds, in comparable proportions, as detected by autoradiography after t.l.c. of an extract prepared in the same way from the faeces of a rat fed with *O*-(hydroxypropyl-2-^{14}C)starch[2]. The major component had an R_F value between those of D-glucose and maltose.

Conventional acetylation of a sample (17 g) of the syrup with anhydrous sodium acetate and acetic anhydride gave a product (18 g), the major component of which had an R_F value (t.l.c.) similar to that of maltotriose hendeca-acetate and was isolated as its amorphous β-anomer by repeated column chromatography.

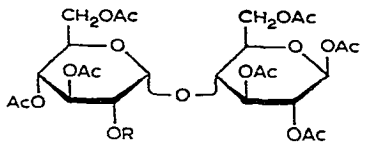
The molecular weight of the acetylated product was determined by osmometry in benzene. The mass and p.m.r. spectra were determined, together with those of a reference compound, β-maltose octa-acetate[3].

RESULTS AND DISCUSSION

The molecular weight of the acetylated compound was 724, indicative of a *O*-(hydroxypropyl)glucosylglucose octa-acetate (mol. wt. 736); this is presumably a maltose derivative, since over 95% of the D-glucose residues in starch are α-(1→4)-linked.

In a preliminary study of the composition of acid hydrolysates of *O*-(hydroxypropyl)starches with a degree of substitution up to 0.18, the hydroxypropyl-monosaccharide fraction was found to contain one major component, which on the basis of its chromatographic behaviour[4] was tentatively identified as 2-*O*-(hydroxypropyl)-D-glucose. In this connection, it is of interest to note that Srivastava *et al.*[5] found that ~84% of the hydroxyethyl groups present in *O*-(2-hydroxyethyl)starch (with a degree of substitution of 0.10) were attached to C-2. Since a 2-*O*-(hydroxypropyl)-D-glucose unit in a starch molecule is likely to block enzymic hydrolysis of the nearest glycosidic bond, one might expect the hydroxypropylglucose moiety of the metabolite to form the non-reducing end of the metabolite, so that the acetylated

metabolite could have structure **1a** or **1b**. Since the hydroxypropyl group contains an asymmetric carbon atom, (*R*)- and (*S*)-forms are likely to be present.



1a R=$CH_2CH(CH_3)OAc$

1b R=$CH(CH_3)CH_2OAc$

2 R=Ac

The relative intensities of the ion peaks in the mass spectra of β-maltose octa-acetate and the acetylated metabolite are shown in Fig. 1. The most-intense peak

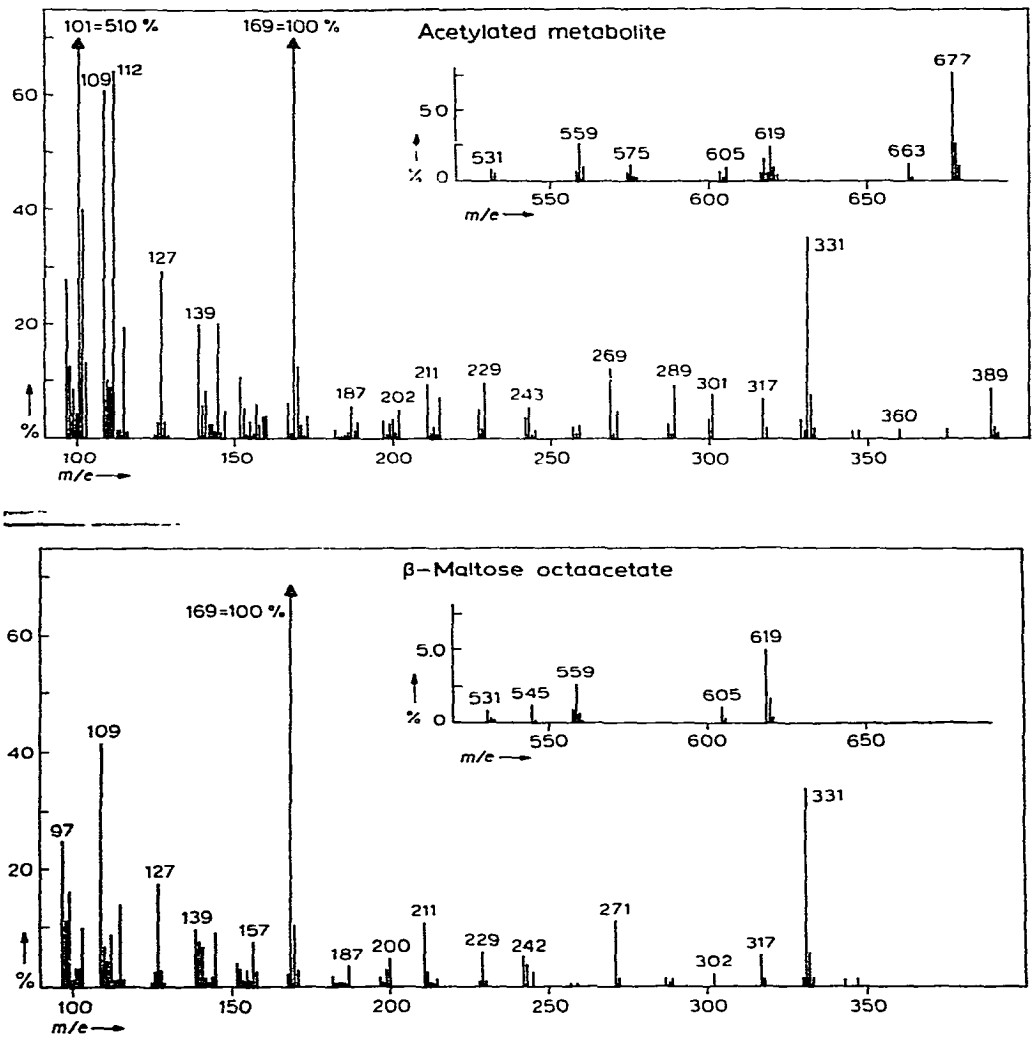
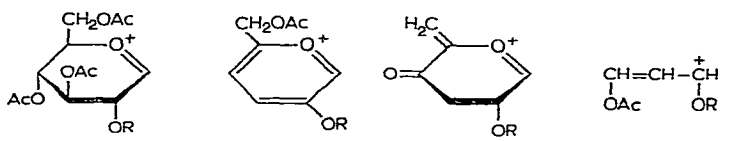


Fig. 1. Mass spectra of the acetylated metabolite and β-maltose octa-acetate. Only peaks greater than 0.5% relative abundance are shown.

(*m/e* 101) in the spectrum of the acetylated metabolite corresponded to the acetoxypropyl fragment ($C_5H_9O_2$; 101.0625; +2.2). The second most-intense peak had the same *m/e* value (169) as the most-intense peak in the spectrum of maltose octa-acetate, and it was therefore adopted as the reference peak to facilitate comparison.

A complete analysis of the spectrum of the acetylated metabolite is not possible because of lack of reference spectra of hydroxypropylated saccharides. However, it appears that fragmentation proceeds mainly according to the schemes proposed by Biemann *et al.*[6,7] for peracetylated pento- and hexo-pyranoses. Molecular ions were not detected, and in the spectrum of maltose octa-acetate the most-intense peak in the higher mass range had *m/e* 619, corresponding to $C_{26}H_{35}O_{17}$, *i.e.* $(M-Ac)^+$. In the spectrum of the acetylated metabolite, the most-intense peak in this range had *m/e* 677, with an elemental composition $C_{29}H_{41}O_{18}$, corresponding to $(M-Ac)^+$ for a fragment of *O*-(hydroxypropyl)maltose octa-acetate. The other peaks in this part of the spectra of the two compounds are apparently due to loss of $-CH_2OAc$ (M−73), −(OAc+HAc) (M−59−60), or −(CH_2OAc+HAc) (M−73−60). The peaks at *m/e* 619 and 559 in the spectrum of the acetylated metabolite may be explained by loss of an acetoxypropyloxy group (+HAc) from the molecular ion, yielding (M−117) and (M−117−60), respectively.

The fragments in the mass range 169–389 appear to be formed for the greater part according to scheme *A* of Biemann *et al.*[6], although fragments of the *B*- and *C*-series are also present. Some typical fragments are shown in Fig. 2.



R=Ac	$C_{14}H_{19}O_9$ (331.1035; +0.6)	$C_{10}H_{11}O_5$ (211.0601; −0.5)	$C_8H_9O_4$[a] (169.0522; +2.1)	$C_7H_9O_4$ (157.0507; +0.7)
R=acetoxypropyl	$C_{17}H_{25}O_{10}$ (389.1474; +2.6)	$C_{13}H_{17}O_6$ (269.1030; +0.5)	$C_{11}H_{15}O_5$ (227.0913; −0.6)	$C_{10}H_{15}O_5$ (215.0917; −0.2)

Fig. 2. Possible structure and elemental composition of some of the ions present in the mass spectra of β-maltose octa-acetate and the acetylated metabolite. Structures adapted from Biemann *et al.*[6]; masses of ion fragments found and deviations in millimass units from masses calculated are given in parentheses. [a]For other structures for this fragment, see Ref. 6.

Of particular interest in connection with the problem of the sequence of the monosaccharide units in the molecule is the peak at *m/e* 302, together with the relative intensities of the peaks at *m/e* 242 and 243 for maltose octa-acetate, and the peak at *m/e* 360, together with the relative intensities of the peaks at *m/e* 300 and 301, for the acetylated metabolite. DeJongh and Biemann[7] detected the peak at *m/e* 302 in the spectrum of methyl α-D-mannopyranoside tetra-acetate but not in the spectrum of D-mannopyranose penta-acetate. They proposed a fragmentation scheme similar to

that in Fig. 3 to explain the formation of the peaks at *m/e* 302, 243, and 242. Apparently, this fragmentation pattern occurs only if HO-1 of an aldose is glycosidically bonded. Therefore, the peak at *m/e* 302 in the spectrum of maltose octa-acetate originates from the non-reducing end of the molecule. Similarly, the presence of the peak at *m/e* 360 in the spectrum of the acetylated metabolite indicates that the hydroxypropylglucose moiety forms the non-reducing end of the molecule.

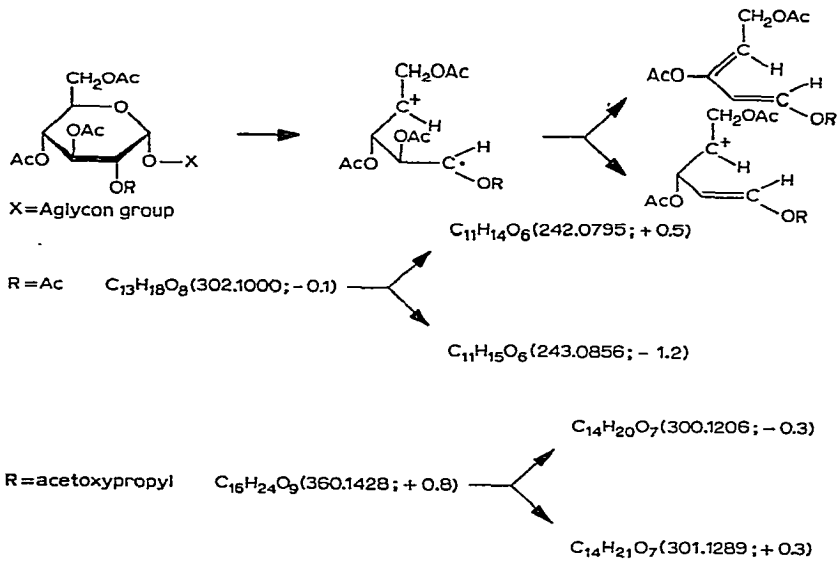


Fig. 3. Possible fragmentation of D-glucopyranosyl glycosides, adapted from DeJongh and Biemann[7]. Masses of ion fragments found and deviations in millimass units from masses calculated are given in parentheses.

In the lower mass range (*m/e* <169), the spectra show differences mainly in the relative intensities of the various peaks, with the exception of the abundant peak at *m/e* 101 in the spectrum of the acetylated metabolite.

The 100-MHz p.m.r. spectral data of the acetylated metabolite (**1**) and β-maltose octa-acetate are shown in Table I and the expanded low-field portion of the spectrum for **1** is shown in Fig. 4; the interpretations are based on recent analyses* by Casu *et al.*[8] and Keilich *et al.*[9].

The ratio of the numbers of protons for the acetylated metabolite is that expected for a β-*O*-(hydroxypropyl)maltose octa-acetate, *viz.* 1 anomeric proton (τ 4.25), 24 acetyl protons (τ ~7.95), 3 methyl protons of the oxypropyl group (τ ~8.8), and 16 other protons (τ 4.5–6.5). Some of the proton absorptions were doubled, *e.g.* those of the β-anomeric proton and the methyl protons of the oxypropyl

*The assignment of the 100-MHz spectrum of β-maltose octa-acetate, based on computer simulations and spin-tickling techniques[10], differed from that of Casu *et al.*[8] as regards the protons in the 6-positions.

TABLE I

COMPARISON OF N.M.R. DATA[a] FOR THE ACETYLATED METABOLITE (1) WITH THOSE OF β-MALTOSE OCTA-ACETATE (2).

Proton position	*Chemical shift (τ)*		*Coupling constants (Hz)*		*Chemical shift differences*	
	1[b]	2[c]	1[b]	2[c]	$\Delta\tau$ (1, 2)[b]	$\Delta\tau$ (1′, 1″)
Reducing moiety						
H-1	4.25	4.24	7.2	7.9	+0.01	0.02
H-2	5.00	5.05	7.3; 7.3	7.9; 8.9	−0.05	0.01
H-3	4.7[d]	4.73	[e]	8.0; 8.9	∼−0.03	[e]
H-4	6.0[d]	6.01	[e]	8.0; 9.6	∼−0.01	[e]
H-5	6.1[d]	6.16	[e]	2.6; 4.6; 9.6	∼−0.06	[e]
H-6	5.48	5.56	2.5; 12.2	2.6; 12.3	−0.08	<0.01
H-6′	5.70	5.76	4.3; 12.2	4.6; 12.3	−0.06	0.02
Non-reducing moiety						
H-1	4.86	4.63	3.5	4.0	+0.23	<0.01
H-2	6.53	5.15	3.5; 10.0	4.0; 10.5	+1.38	<0.01
H-3	4.7[d]	4.65	[e]	9.5; 10.5	∼+0.05	[e]
H-4	5.09	4.98	8.0; 9.3	9.5; 9.6	+0.11	0.01
H-5	6.1[d]	5.99[d]	[e]	2.5; 4.5; 9.6	∼+0.11	[e]
H-6	5.80	5.79	5.4; 12.6	4.5; 12.3	+0.01	<0.01
H-6′	5.96	5.92	1.8; 12.4	2.5; 12.3	+0.04	<0.01
Acetoxypropyl group						
CH	5.06[d]		[e]			<0.01
CH_2	6.44[c,f]		5.0; 5.6; 10.6			0.01
CH_3	8.81		6.5			0.04

[a]100-MHz spectra in $CDCl_3$ at 70°. [b]Average value for 1′ and 1″. [c]Computer-simulated assignment. [d]Preliminary assignment. [e]Not determined. [f]In one isomer appears as an AB part of an ABC system, with $\Delta\tau$(A, B) = 0.18 p.p.m.; the other gives rise to a doublet.

group and, under optimum conditions, 16 acetoxy methyl absorptions were observed. The differences in chemical shift of corresponding signals were found to be <0.05 p.p.m. and reflect the presence of approximately equimolar amounts of the (*R*)- and (*S*)-forms of the acetoxypropyl group (see subsequent discussion). Average values of chemical shifts and coupling constants are given in Table I.

The replacement of an acetoxy group by an acetoxypropyloxy group should result in an upfield shift of 1.3–1.4 p.p.m. of the proton attached to the substituted carbon atom. Despite the complexity of the spectrum of the acetylated metabolite, H-1 of the non-reducing D-glucose moiety could be recognized as a doublet (τ 4.86) with a single, *gauche* coupling constant of 3.5 Hz. Spin decoupling at this frequency resulted in a collapse of a quartet centered at τ 6.53 (*J* 3.5 and 10.0 Hz) to a doublet with *J* 10.0 Hz. This indicates that H-2 of the non-reducing end of the molecule absorbs ∼1.4 p.p.m. upfield with respect to the corresponding proton in β-maltose octa-acetate. Therefore, the acetoxypropyloxy group is attached to C-2 of the non-reducing moiety of the acetylated metabolite.

The chemical shifts of all other pairs of corresponding protons in the acetylated

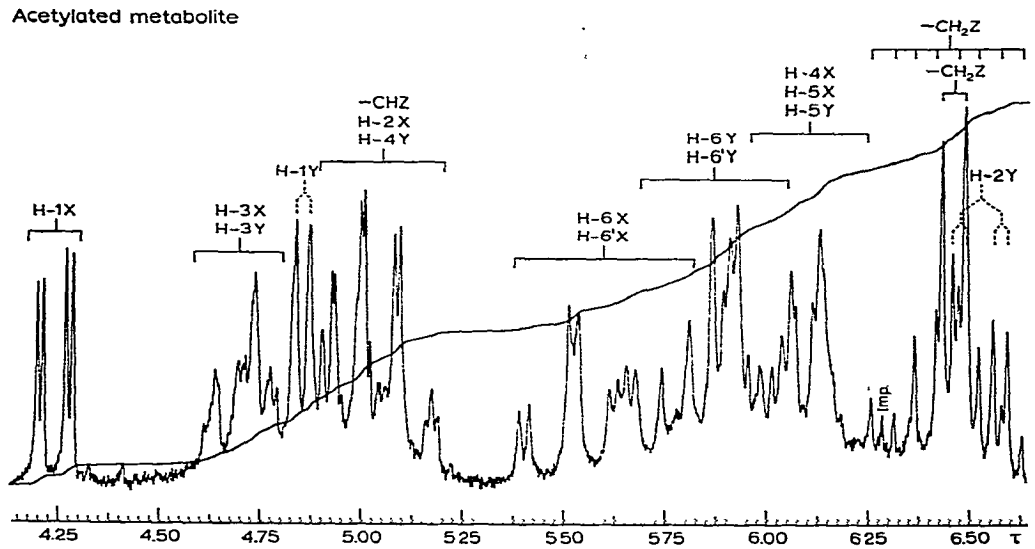


Fig. 4. The expanded low-field portion of the 100-MHz spectrum of the acetylated metabolite in chloroform-*d* at 70°. The protons of the reducing D-glucopyranose residue are indicated by the suffix X, those of the non-reducing unit by the suffix Y, and those of the hydroxypropyl moiety by the suffix Z.

metabolite and β-maltose octa-acetate were similar (Table I). The chemical shift (τ 5.06) assigned to the methine proton of the acetoxypropyloxy group is typical of an aliphatic C_2CHOAc group; a methine proton in an ether function of the type C_2CHOR would be expected to absorb near τ 6.4. The acetylated metabolite therefore contains a 2-acetoxypropyl group, *i.e.* structure **1a**. Consistent with this conclusion is the assigned chemical shift (τ 6.44) of the methylene protons of the 2-acetoxypropyloxy group (expected value τ 6.4). The latter assignment was confirmed by a computer simulation, using the parameters of Table I.

The assignments in Table I were confirmed by the usual spin-decoupling experiments and studies with paramagnetic shift reagents[11], and the structure of the acetylated metabolite was concluded thereby to be the β-anomer of 4-*O*-[2-*O*-(2-hydroxypropyl)-α-D-glucopyranosyl]-D-glucopyranose octa-acetate (**1a**).

With regard to isomerism at the acetoxypropyl group in **1**, the following points may be noted. It is well known that epimeric acetylated carbohydrates differ considerably in chemical shifts of the epimeric and other ring protons[12]. The small (<0.05 p.p.m.) differences in the chemical shift of the ring protons in the two isomers (**1′** and **1″**) of **1** (Table I) exclude the possibility of epimers. Molecular models show that the structure **1**, even with the 2-acetoxypropyl group, is not strongly congested. Therefore, the occurrence of two isomers due to hindered rotation is unlikely. Moreover, heating of the sample from room temperature to 80° did not lead to broadening or disappearance of the two sets of absorptions. On the contrary, the signals became sharper at higher temperatures. In the reaction between propylene oxide and starch,

nearly equivalent amounts of (*R*)- and (*S*)-hydroxypropyl derivatives are to be expected; it has been established that such isomers yield, in principle, different p.m.r. spectra[13].

The above data and argument show that the acetylated metabolite is 4-*O*-{2-*O*-[(*RS*)-2-hydroxypropyl]-α-D-glucopyranosyl}-D-glucopyranose octa-acetate. It seems unlikely that the metabolite was originally excreted as a partially acetylated product or that a substituent was removed by the treatment with acetic anhydride. Therefore, it may be concluded that, in the rat, the major metabolite of *O*-(hydroxypropyl)starches, at least of products with a degree of substitution below 0.11, is 4-*O*-{2-*O*-[(*RS*)-2-hydroxypropyl]-α-D-glucopyranosyl}-D-glucopyranose.

The *O*-(hydroxypropyl)starches used in the present study were prepared[1] by treating potato starch with propylene oxide in aqueous alkali. The p.m.r. data showed that the major metabolite is an *O*-(2-hydroxypropyl) derivative. By contrast, Ilg *et al.*[4] found only *O*-(1-hydroxypropyl)-D-glucoses in a hydrolysate of a hydroxypropylstarch, prepared by treating corn starch with propylene oxide in methanol and sodium methoxide as a catalyst. This indicates that the reaction conditions may have a distinct effect on the opening of the epoxide ring.

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