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Note

Stereoselective hydrolysis of radiolabelled *O*-pivaloyl derivatives of methyl 2-acetamido-2-deoxy-D-glucopyranoside*

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There have been many studies of carboxylesterases present in the sera[1,2] and tissues[2,3] of animals. Esters are often used as prodrugs[4,5]. Some tumour promoters are esters that are deactivated by esterases[1,3,6]. Furthermore, some anti-tumour agents are esters and the esterase activity of various tissues must be taken into account in chemotherapy[7].

Esterases have been also used in synthesis, *e.g.*, hog-liver carboxylesterase hydrolyses asymmetric chiral and prochiral esters[8,9] and can induce stereospecific transesterifications[10]. A lipase from *Candida cylindracea* can remove selectively the 6-*O*-acyl group from acylated methyl glucopyranosides[11] at rates that depend on the anomeric configuration and the type of the acyl substituent.

We now report on the synthesis of ^{14}C-labelled methyl 2-acetamido-2-deoxy-3,6-di-*O*-pivaloyl-α- and -β-D-glucopyranosides and their susceptibility to esterases from rabbit serum. The pivaloyl group, which has been used in selective acylations of sugars[12], does not migrate readily and is easily detectable by ^{1}H-n.m.r. spectroscopy. The conventional saponification of the pivaloyl group with methanolic sodium methoxide proceeds slowly, but this limitation was overcome by using esterases from mammalian sera[2,13,14] and liver[2] for deacylations under mild conditions.

In a one-pot synthesis, convenient for radiolabelled compounds, 2-acetamido-2-deoxy-D-[1-^{14}C]glucopyranose was treated with methanol in the presence of a cation-exchange resin to give an ~4:1 α,β-mixture of methyl glycosides (**1**, 95% based on t.l.c. data). The crude product was treated with 3 equiv. of pivaloyl chloride in pyridine for 16 h at ambient temperature, to give a mixture of the 3,6-dipivalates **4**α and **4**β, together with some 6-pivalate **2**α and methyl 2-acetamido-2-deoxy-3,4,6-tri-*O*-pivaloyl-α- and -β-D-[1-^{14}C]gluco-

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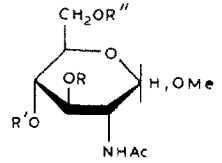
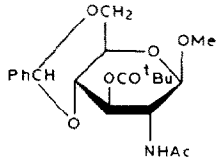


1 R = R′ = R″ = H
2 R = R′ = H, R″ = ^{t}BuCO
3 R = ^{t}BuCO, R′ = R″ = H
4 R = R″ = ^{t}BuCO, R′ = H
5 R = R′ = Ac, R″ = ^{t}BuCO
6 R = ^{t}BuCO, R′ = R″ = Ac



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pyranoside. These products were isolated by column chromatography and identified by comparison with their unlabelled analogues[12].

The radiolabelled 3,6-dipivalates **4α** and **4β** were used as substrates for the esterase from rabbit serum. De-esterifications proceeded in a stepwise manner and the ratio of products depended on the time of reaction and the anomeric configuration of the substrate (Table I). Thus, **4α** gave 78% of the 3-ester (**3α**) after only 5 min, with traces of the 6-ester **2α**. After 20 min, virtually all **4α** had reacted, giving 88% of **3α** with traces of other products. This ratio did not change after 1 h and 3 h, but, after 6 h, a second deacylation step had occurred, involving the hydrolysis of **3α** to **1α** (11%). After 24 h, 37% of **1α** was obtained and 55% of **3α** remained.

Enzymic de-esterification of **4β** was much slower than that of **4α**. After 5 min, 88% of **4β** remained and, after 20 min, a 1:3 mixture of mono-esters **2β** and **3β** was formed, with 73% of **4β** unreacted. Incubation for 1 h produced a 1:5 mixture of **2β** and **3β** (63% of **4β**), and for 3 h a 1:10 mixture of **2β** and **3β** (27%

TABLE I

ENZYMIC DEPIVALOYLATION OF **4α** AND **4β**: EFFECT OF REACTION TIME AND ANOMERIC CONFIGURATION

Time	*Products*[a]							
	1α[b]	**2α**[c]	**3α**[c]	**4α**[d]	**1β**[b]	**2β**[c]	**3β**[c]	**4β**[d]
0				100				100
5 min	1	2	78	18		4	7	88
20	1	2	88	7	1	6	19	73
1 h	2	4	88	5	1	6	29	63
3	2	4	89	4	3	6	63	27
6	11	4	81	4	9	3	69	18
24	37	4	55	3	30	14	44	12

[a]Analysis based on radioactivity and given as a percentage of the total recovered after t.l.c. [b]Methyl 2-acetamido-2-deoxy-α- or -β-D-[1-^{14}C]glucopyranoside. [c]3-Pivalate and 6-pivalate (α- or β-). [d]Methyl 2-acetamido-2-deoxy-3,6-di-*O*-pivaloyl-α- or -β-D-[1-^{14}C]glucopyranoside.

of **4β**). After 6 h, 9% of **1β** was present, but the main product was a 1:23 mixture of **2β** and **3β**; after 24 h, 30% of **1β** was present.

Thus, although preferential cleavage of the 6-ester group occurs in **4α** and **4β**, the former yields the 3-ester **3α** almost exclusively, whereas de-esterification of **4β** is slower and yields the 3-ester **3β** together with a small amount of the 6-ester **2β**. The degree of stereoselectivity for **4β** is highest after reaction for 6 h. Steric hindrance associated with the proximity of MeO-1 and the 6-ester group in **4β** may be the cause of the slower reaction of the 6-pivalate group compared to that in **4α**. Prolongation of reaction times favors 3-deacylation, leading to a lower degree of stereoselectivity in the β-anomer.

Stereoselective enzymic hydrolysis with other pivaloylated sugars has been reported. Thus, for methyl 2,6-di-*O*-pivaloyl-α-D-glucopyranoside, the 6-ester group is removed much more readily than the 2-ester group[14]. Pig-liver esterase has been used to hydrolyse prochiral diesters to give chiral monoesters with large enantiomeric excess[8,9,15], and also for the preparation of chiral δ-lactones[16] and amino acids[17] from prochiral diesters.

All of the products of enzymic hydrolysis reported here were identified by comparison (t.l.c.) with their unlabelled, independently synthesized analogues. Whereas the unlabelled 3,6-diesters (**4α** and **4β**) and the 6- (**2α**) and 3-esters (**3α**) have been described[12], the 6- (**2β**) and 3-ester (**3β**) were unknown hitherto. Thus, treatment of methyl 2-acetamido-2-deoxy-D-glucopyranoside (**1**) with ~1 equiv. of pivaloyl chloride in pyridine at room temperature produced an ~4:1 α,β-mixture of the 6-pivalates **2**. Column chromatography then gave **2α**[12] and **2β**. The structure of **2β** was determined on the basis of analytical and ^{1}H-n.m.r. data for its 3,4-diacetate **5β**.

In order to obtain the 3-pivalate **3β**, methyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-β-D-glucopyranoside[18] was treated with an excess of pivaloyl chloride for 24 h at 70°, and 14% of **7** was obtained. Treatment of **7** with hot dilute acetic acid produced the 3-ester **3β**, which was characterized as its 4,6-diacetate **6β**.

EXPERIMENTAL

General methods. — Melting points are uncorrected. Optical rotations were determined for 1% solutions in chloroform or methanol. Column chromatography was performed on silica gel (Merck), and t.l.c. on Kieselgel G (Merck), using *A*, ethyl acetate–benzene (2:1); *B*, ethyl acetate–methanol (5:1); and *C*, ethyl acetate–benzene–ethanol (2:2:1). Detection was effected by charring with sulphuric acid. ^{1}H-N.m.r. spectra (100 MHz, CD_3OD or $CDCl_3$, internal Me_4Si) were recorded with a Jeol JNM FX-100 F.t. spectrometer. Radioactivity was measured by using a Beckman LS-100 C liquid scintillation counter and Aquasol (NEN) as a scintillation cocktail.

*Methyl 2-acetamido-2-deoxy-*D*-[1-^{14}C]glucopyranoside* (**1**) *and its pivaloylation.* — A solution of 2-acetamido-2-deoxy-D-[1-^{14}C]glucopyranose (0.925 MBq)

and 2-acetamido-2-deoxy-D-glucopyranose (44 mg, 0.2 mmol) in dry methanol (2.6 mL) was boiled under reflux for 4 h in the presence of Amberlite IR-120 (H^+) resin. The resin was collected and washed with warm methanol (6 × 1 mL), and the combined filtrate and washings were concentrated under reduced pressure to give amorphous **1** (44.5 mg, 95%). T.l.c. (solvent *B*) indicated **1** to be a ~4:1 α,β-mixture (based on radioactivity).

A solution of crude **1** (44.5 mg, 0.18 mmol) in dry pyridine (1 mL) was treated with pivaloyl chloride (0.075 mL) at room temperature for 4 h. More pivaloyl chloride (0.05 mL) was then added and the reaction was continued for 12 h. Ethanol was added, the solvents were evaporated under reduced pressure, and toluene was distilled (5×) from the residue in order to remove traces of pyridine. Column chromatography (solvent *A*), repeated twice, yielded chromatographically homogeneous methyl 2-acetamido-2-deoxy-3,4,6-tri-*O*-pivaloyl-α-D-[1-^{14}C]glucopyranoside (7.8 mg, 8%), R_F 0.65 (solvent *A*), 5.2 MBq/mmol. Eluted second was the α-anomer **4α** (19.3 mg, 30%), R_F 0.63, 5.8 MBq/mmol. Further elution gave the β-anomer of the 3,4,6-tripivalate (6 mg, 6%), R_F 0.57, 5.2 MBq/mmol; then the 3,6-dipivalate **4β** (7.5 mg, 7%), R_F 0.45, 5.2 MBq/mmol.

Further elution with solvent *B* produced a 2:1 mixture (24.5 mg, 30% based on radioactivity) of the 3,6-dipivalate **4β** and 6-pivalate **2α**.

All products were identified by comparison (t.l.c., solvents *A*–*C*) with authentic unlabelled compounds.

*Enzymic deacylations of methyl 2-acetamido-2-deoxy-3,6-di-*O*-pivaloyl-α-(**4α**) and -β-D-[1-^{14}C]glucopyranoside* (**4β**). — Each incubation mixture contained 0.1M phosphate-buffered saline (PBS, 75 μL, pH 7.2), rabbit serum (50 μL), and methyl sulfoxide (10 μL) containing **4α** or **4β**. The resulting mixtures (135 μL) were mM with respect to **4**. Incubations were allowed to proceed for 5 and 20 min, 1, 3, 6, and 24 h (Table I) at 37° and were stopped by the addition of ethanol (0.6 mL). Serum proteins were removed by centrifugation (1500*g*, 15 min). Each supernatant solution was concentrated under reduced pressure, and an aliquot (30 μL) of a solution of the residue in aqueous 50% ethanol (100 μL) was subjected to t.l.c. (solvent *C*) with unlabelled **1α**–**4α** or **1β**–**4β** as references. R_F values: **1α**, 0.15; **2α**, 0.39; **3α**, 0.53; **4α**, 0.76; **1β**, 0.08; **2β**, 0.34; **3β**, 0.42; **4β**, 0.70. The radioactivity was associated only with the expected products.

The reference compounds were detected by charring with sulphuric acid. The absorbent containing each radiolabelled product (**1α**–**4α**, or **1β**–**4β**) of enzymic action was collected and suspended in aqueous 75% ethanol (2 mL) and a scintillation cocktail (2 mL). The radioactivity is expressed as a percentage (Table I), and the recovery was 98–100%.

Each enzymic deacylation was performed at least twice and the results were 96% reproducible.

T.l.c. and determinations of radioactivity were repeated 2–3 times, the reproducibility being 96–98%.

*Methyl 2-acetamido-2-deoxy-6-*O*-pivaloyl-β-D-glucopyranoside* (**2β**). — A

solution of methyl 2-acetamido-2-deoxy-D-glucopyranoside (**1**; 1 g, 4.25 mmol; α,β-ratio ~5:1) in dry pyridine (10 mL) was treated with pivaloyl chloride (0.6 mL) at room temperature for 40 min. Ethanol was added, the solvents were removed under reduced pressure, and column chromatography (solvent *A*, then solvent *C*) of the residue gave methyl 2-acetamido-2-deoxy-6-*O*-pivaloyl-α-D-glucopyranoside[12] (**2**α; 456 mg, 34%), followed by **2**β (117 mg, 9%), isolated as a solid, $[\alpha]_D$ −10° (methanol). ^{1}H-n.m.r. data (CD_3OD): δ 3.46 (s, OMe), 2.07 (s, NAc), 1.31 (s, PivO-6).

Conventional acetylation of **2**β (50 mg, 0.157 mmol) with acetic anhydride–pyridine gave, after column chromatography (solvent *C*), the 3,4-diacetate **5**β, isolated as a solid (50 mg, 79%), $[\alpha]_D$ ~0° (chloroform). ^{1}H-N.m.r. data ($CDCl_3$): δ 4.38 (d, $J_{1,2}$ 9.96 Hz, H-1), 3.41 (s, OMe), 2.05, 2.03, (2 s, 2 AcO), 1.95 (s, NAc), 1.21 (s, PivO-6).

Anal. Calc. for $C_{18}H_{29}NO_9$: C, 53.59; H, 7.25; N, 3.47. Found: C, 53.62; H, 7.45; N, 3.57.

Methyl 2-acetamido-2-deoxy-3-O-pivaloyl-β-D-glucopyranoside (**3**β). — A solution of methyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-D-glucopyranoside[18] (1 g, 3.09 mmol; α,β-ratio ~5:1) in dry pyridine (7 mL) was treated with pivaloyl chloride (1.5 mL) for 24 h at 70°. Ethanol (5 mL) was added, the solvents were removed under reduced pressure, and column chromatography (solvent *A*) of the residue gave methyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-3-*O*-pivaloyl-α-D-glucopyranoside[12] (397 mg, 31.5%), R_F 0.59 (solvent *A*), followed by the β-anomer **7** (170 mg, 13.5%), R_F 0.42 (solvent *A*). Crystallization from aqueous 50% ethanol gave material having m.p. 185–187°, $[\alpha]_D$ −94° (chloroform). ^{1}H-N.m.r. data ($CDCl_3$): δ 7.44–7.26 (m, 5 H, Ph), 5.51 (s, PhC*H*), 3.28 (s, OMe), 1.94 (s, NAc), 1.21 (s, PivO-3).

Anal. Calc. for $C_{21}H_{28}NO_7$: C, 61.90; H, 7.17; N, 3.44. Found: C, 61.65; H, 7.41; N, 3.28.

A solution of **7** (170 mg, 0.417 mmol) in acetic acid (6 mL) and water (3 mL) was heated at 100° for 1 h, and co-concentrated with toluene and water (3×). A solution of the crude product in water was washed with ethyl acetate, then concentrated under reduced pressure to give chromatographically pure **3**β (102 mg, 77%) as a solid, $[\alpha]_D$ −31° (methanol). ^{1}H-N.m.r. data (CD_3OD): δ 3.51 (s, OMe), 1.97 (s, NAc), 1.24 (s, PivO-3).

Treatment of **3**β (100 mg, 0.313 mmol) with acetic anhydride–pyridine for 16 h at ambient temperature gave, after column chromatography (solvent *C*), amorphous methyl 2-acetamido-4,6-di-*O*-acetyl-2-deoxy-3-*O*-pivaloyl-β-D-glucopyranoside **6**β (137 mg, 82%). Crystallization from ethyl acetate–light petroleum gave material having m.p. 97–100°, $[\alpha]_D$ −10° (chloroform). ^{1}H-N.m.r. data ($CDCl_3$): δ 5.55 (d, *J* 9.55 Hz, NH), 4.46 (d, $J_{1,2}$ 8.20 Hz, H-1), 3.49 (s, OMe), 2.10 (s, AcO-6), 2.00 (s, AcO-4), 1.93 (s, NAc), 1.14 (s, PivO-3).

Anal. Calc. for $C_{18}H_{29}NO_9$: C, 53.59; H, 7.25; N, 3.47. Found: C, 53.67; H, 7.38; N, 3.50.

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