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Note

Selective deacylation on the glucosyl moiety of octa-*O*-acetylsucrose by enzymic hydrolysis: formation of 2,1′,3′,4′,6′-penta-*O*-acetylsucrose

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Partially acetylated sucroses have been prepared by several approaches. They can be synthesized, from trityl ethers and acetals of sucrose, as precursors, by several conventional chemical methods[1–3]. They can also be prepared by the selective deacylation of peracetylated sucrose, in chloroform solution on aluminium oxide[4,5], in methanol solution on aluminium oxide impregnated with potassium carbonate[6,7], dissolved in propylamine or isopropylamine[8], or by enzymic hydrolysis[9]. However, all of these methods usually cause deacylation in the fructosyl moiety. Here we report that octa-*O*-acetylsucrose (**1**) was hydrolyzed by lipase OF (*Candida cylindracea*); it was found that deacylation occurred only in the glucosyl moiety of **1**.

The hydrolysis of octa-*O*-acetylsucrose (**1**) with lipase OF was carefully studied. The major products at different reaction times were isolated. Their structures were analyzed and the pathway of hydrolysis of **1** by lipase OF was proposed in Scheme 1. In our previous reports[10], it was shown that lipase OF preferentially cleaved the 6-acetate to form 2,3,4,1′,3′,4′,6′-hepta-*O*-acetylsucrose (**2**) which rearranged non-enzymically to 2,3,6,1′,3′,4′,6′-hepta-*O*-acetylsucrose (**3**) through 4 → 6 acyl migration[1]. We now find that the enzyme again attacks the 6-acetate group of **3** to produce 2,3,1′,3′,4′,6′-hexa-*O*-acetylsucrose (**4**). A small amount of 2,6,1′,3′,4′,6′-hexa-*O*-acetylsucrose (**5**) accompanying **4** was found by analysis of NMR spectra and was isolated by HPLC. Since **4** is stable for 120 h in the phosphate buffer, without any acetyl migration, it is believed that **5** is formed by the direct deacylation of the 3-acetate of **3** and not from **4** by 3 → 6 acetyl migration. Finally, the major pentaacetate, 2,1′,3′,4′,6′-penta-*O*-acetylsucrose (**6**), was obtained by deacy-

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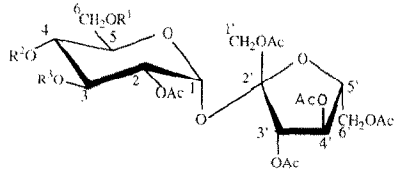
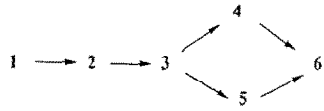


	R^1	R^2	R^3
1	Ac	Ac	Ac
2	H	Ac	Ac
3	Ac	H	Ac
4	H	H	Ac
5	Ac	H	H
6	H	H	H



Scheme 1. Hydrolysis of sucrose octaacetate by lipase OF.

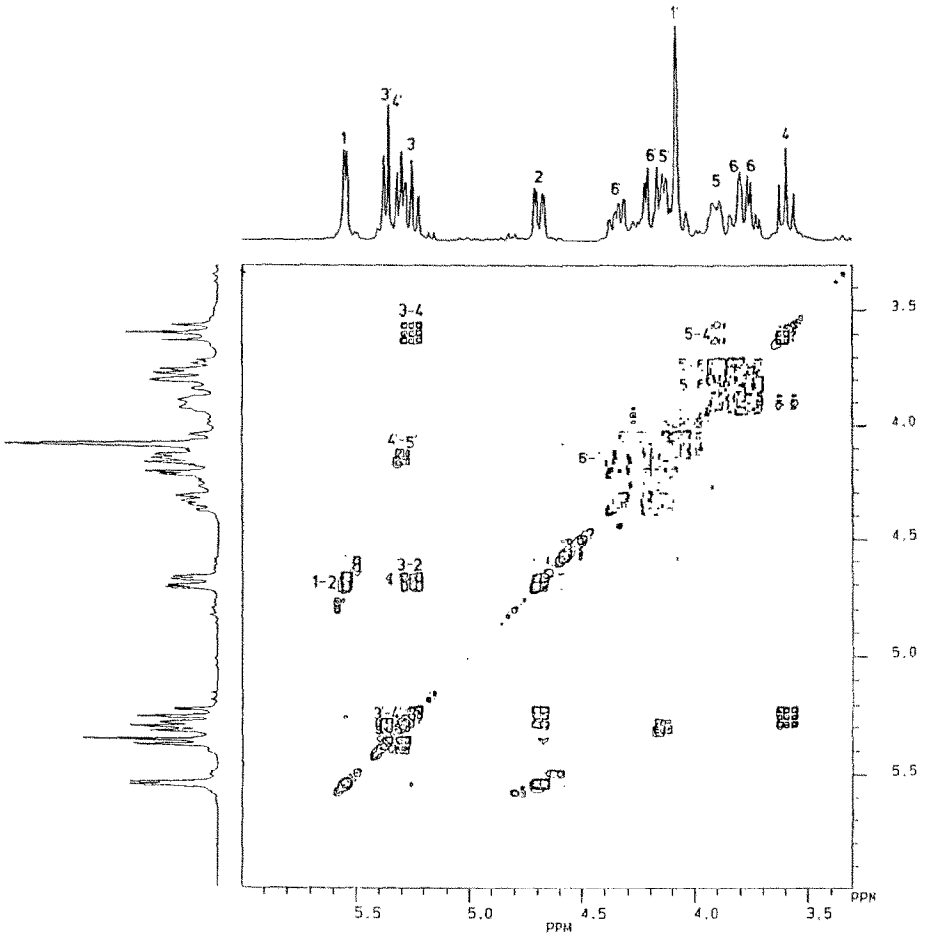


Fig. 1. Two-dimensional ^1H–^1H COSY spectrum of **4**.

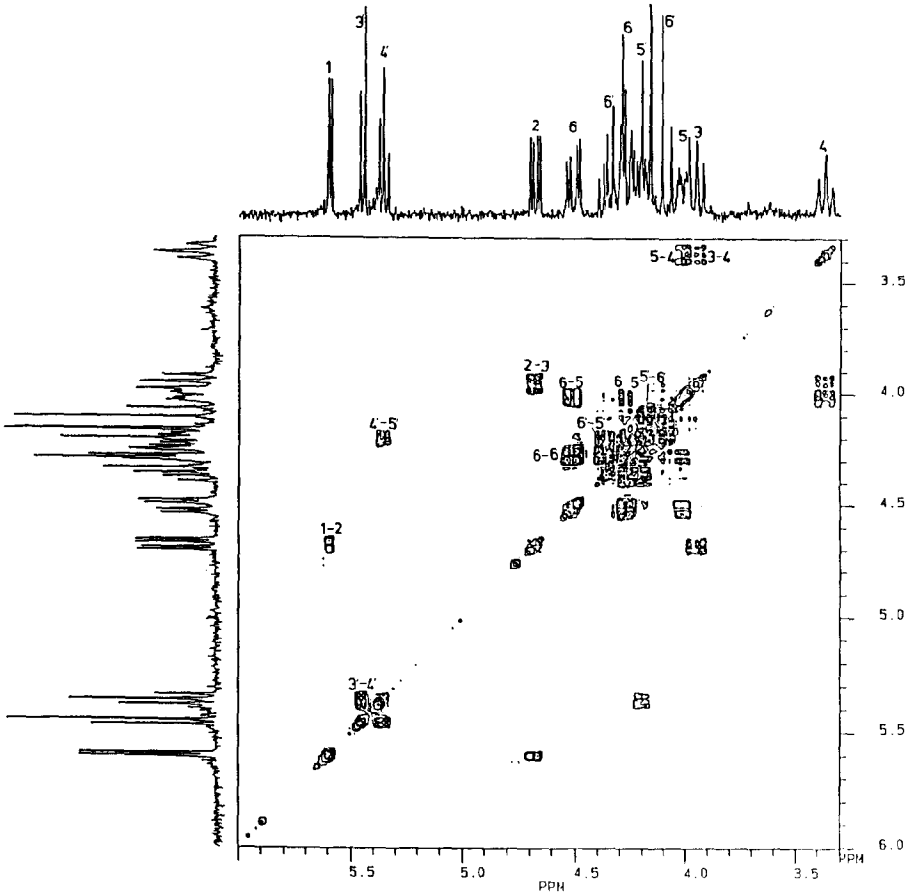


Fig. 2. Two-dimensional ^1H–^1H COSY spectrum of **5**.

lating the 6-acetate of **5** or the 3-acetate of **4**. The yields of these products are various, depending on the reaction time.

The structures of the products obtained from the hydrolysis of **1** by lipase OF were determined, mainly based on the chemical shifts of their ^1H NMR spectra in comparison with those of **1**[11] and other partially acetylated sucroses which have been published. The 2D COSY NMR spectra of compounds **4**, **5**, and **6** are shown in Figs. 1, 2, 3, and 4, respectively, and their chemical shifts and coupling constants are also listed in Tables I and II.

Compound **1** contains three primary esters and five secondary esters. According to the previous reports[6,7,9,10,12,13], the ester groups in the furanose ring are more reactive towards deacylation than those in the pyranose ring, and all the acetate groups in **1** except the 3-acetate have been reported to be deacylated by chemical or enzymic methods. This is the first time that selective deacylation by enzymic hydrolysis of the 3-acetate of **1** has been demonstrated.

EXPERIMENTAL

Lipase OF (*Candida cylindracea*) was purchased from Meito-Sangyo Co., Ltd., Japan and was used for hydrolytic reactions without further purification. Thin-layer chromatography was performed on Silica Gel 60 G (Merck) precoated on aluminum sheets. A HPLC system of Gilson, Inc. (France) was used for the analytical separations, which consisted of one 302 pump, one 305 pump, a 811B dynamic mixer, a 805 manometric module, and a model 7125 syringe loading sample injector (Rheodyne Inc.), coupled to a 115 variable-wavelength UV spectrophotometer and a Macintosh SE personal computer with Dynamax HPLC methods manager software (version 1.2) as an integrator. Optical rotations were measured on a Polartronic Universal Polarimeter (Schmidt & Haensch). ^{1}H NMR and ^{13}C NMR spectra were recorded with a 300-MHz Bruker instrument. All chemical

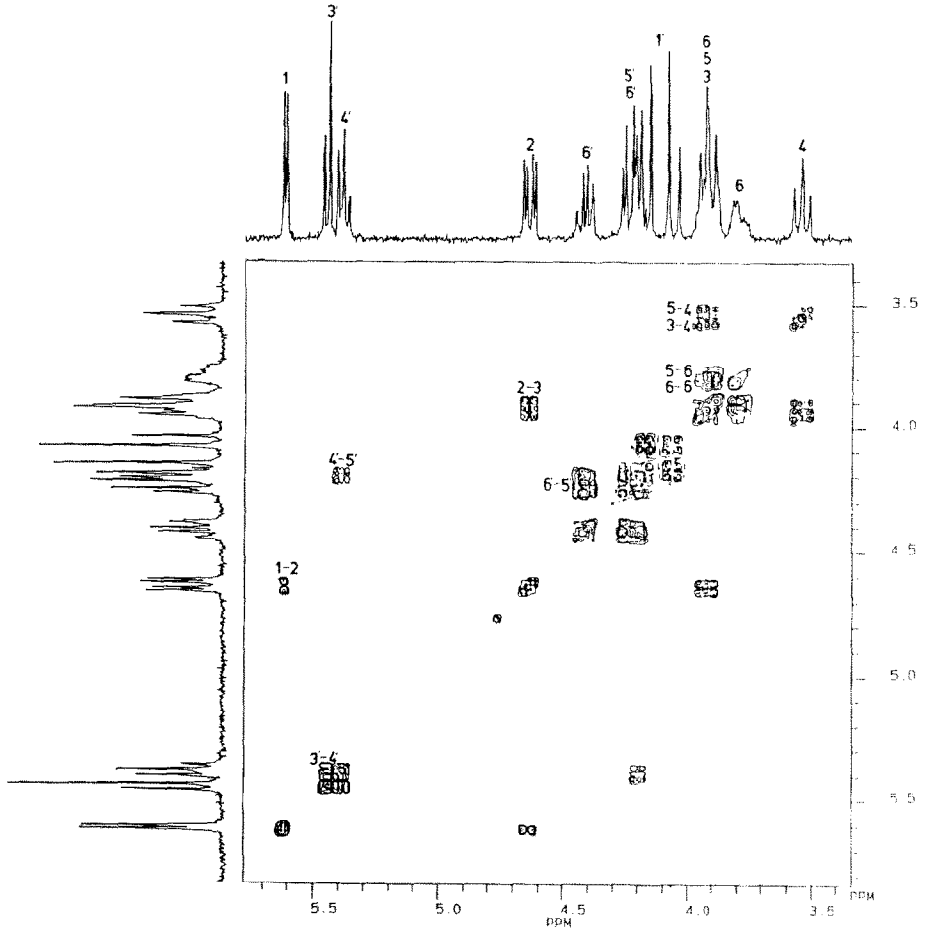


Fig. 3. Two-dimensional ^{1}H–^{1}H COSY spectrum of **6**.

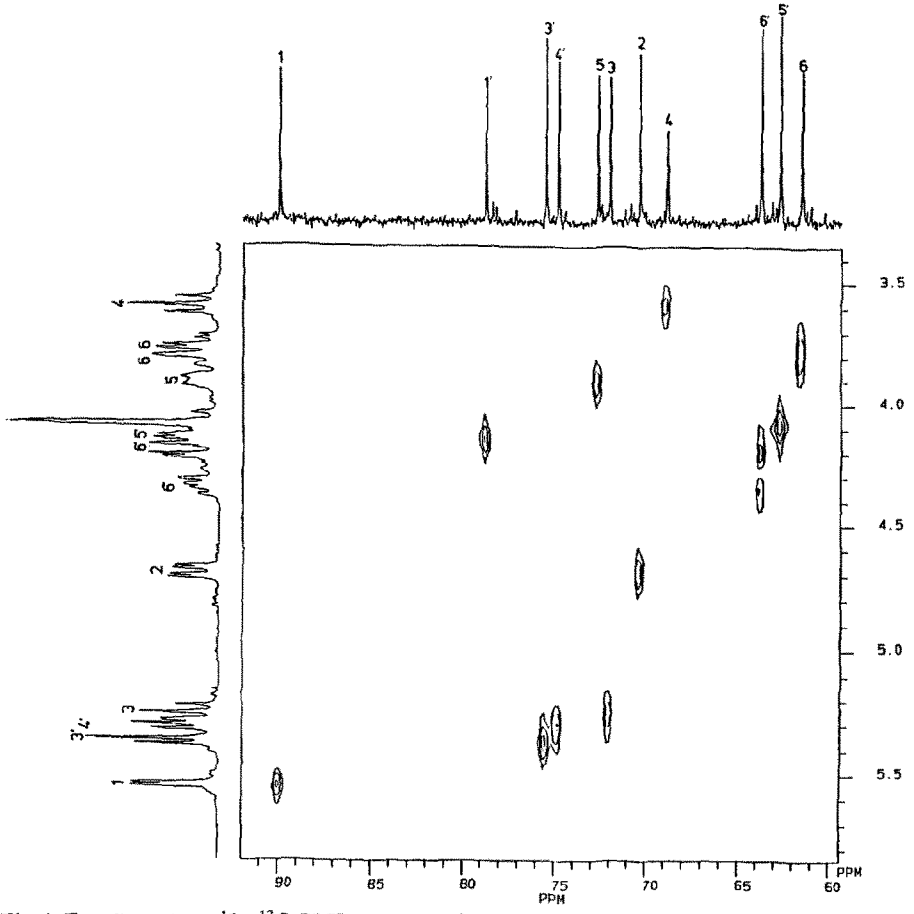


Fig. 4. Two-dimensional ^{1}H–^{13}C COSY spectrum of **4**.

shifts are reported in ppm, using Me_4Si as internal standard. Organic solvents were reagent grade. The substrate, octa-*O*-acetylsucrose (**1**), was synthesized by an established method[14], and its ^{1}H and ^{13}C NMR spectra agreed with those published.

Enzymic hydrolysis of sucrose octaacetate.—To a solution of 5 g (7.35 mmol) of **1** in 300 mL of phosphate buffer (pH 7.0, 0.1 M, containing 0.2 M NaCl and 3 mM $CaCl_2$) was added 15 g of lipase OF (*Candida cylindracea*, Meito-Sangyo Ltd., Japan). The mixture was stirred at room temperature. The progress of the reaction was monitored by TLC with 1:50 MeOH–ether as the developing system. Visualization was by spraying with 5% H_2SO_4 in EtOH, then heating at 110°C for 10 min. When the accumulation of hexa-*O*-acetylsucrose and penta-*O*-acetylsucrose was evident by TLC (reaction time, 72 h), the reaction was stopped by extracting the products with $CHCl_3$. The extract was evaporated under reduced pressure and

TABLE I

^{1}H NMR chemical shifts and coupling constants of **4**, **5**, and **6** [a]

Atom	**4**	**5**	**6**
H-1	5.54 (d, $J_{1,2}$ 3.5)	5.60 (d, $J_{1,2}$ 3.7)	5.57 (d, $J_{1,2}$ 3.7)
H-2	4.68 (dd, $J_{2,3}$ 10.3)	4.68 (dd, $J_{2,3}$ 10.2)	4.66 (dd, $J_{2,3}$ 10.1)
H-3	5.25 (t, $J_{3,4}$ 9.6)	3.95 (t, $J_{3,4}$ 9.5)	3.80–3.91
H-4	3.59 (t, $J_{4,5}$ 9.5)	3.36 (t, $J_{4,5}$ 9.5)	3.58 (t, $J_{4,5}$ 9.1)
H-5	3.86–3.92 (m)	3.98–(4.03) (m)	3.80–3.91 (m)
H-6a	3.75–3.80	4.51 (dd, $J_{5,6a}$ 4.2, $J_{6a,6b}$ 12.3)	3.80–3.91
H-6b	3.73 (dd, $J_{5,6b}$ 4.7, $J_{6a,6b}$ 12.5)	4.22–4.27	3.80–3.91
H-1′	4.12–4.16	4.13 (dd $J_{1'a,1'b}$ 12.2)	4.11 (dd $J_{1'a,1'b}$ 12.1)
H-3′	5.36 (d, $J_{3',4'}$ 5.8)	5.45 (t, $J_{3',4'}$ 6.6)	5.44 (t, $J_{3',4'}$ 6.6)
H-4′	5.32 (t, $J_{4',5'}$ 5.8)	5.35 (t, $J_{4',5'}$ 6.3)	5.29 (t, $J_{4',5'}$ 6.4)
H-5′	4.12–4.16 (m)	4.14–4.21 (m)	4.14–4.22 (m)
H-6′a	4.35 (dd, $J_{5',6'a}$ 7.3 $J_{6'a,6'b}$ 12.2)	4.36 (dd, $J_{5',6'a}$ 7.2, $J_{6'a,6'b}$ 11.9)	4.38 (dd, $J_{5',6'a}$ 7.0, $J_{6'a,6'b}$ 11.6)
H-6′b	4.19 (dd, $J_{5',6'b}$ 3.4)	4.06–4.14	4.23 (dd, $J_{5',6'b}$ 4.3)
$COOCH_3$	1.93–2.13 (m)	2.06–2.15 (m)	2.07–2.21 (m)

[a] The units of chemical shift and coupling constants are ppm and Hz, respectively.

the products were purified on a column of silica gel eluted with a gradient of 1:100 → 4:100 MeOH–ether.

Separation of **4** *and* **5** *by HPLC.*—Compounds **4** and **5** were separated on a Vydac C_{18} column at ambient temperature isocratically with 1:3 acetonitrile–H_2O (by volume), at a flow rate of 2.0 mL/min, and UV detection (214 nm). The retention times of **4** and **5** were 23.93 and 24.88 min, respectively. The following compounds were obtained.

TABLE II

^{13}C NMR chemical shifts of **4**, **5**, and **6** [a]

Atom	**4**	**5**	**6**
C-1	89.99	90.30	90.21
C-2	70.38	70.51	71.23
C-3	72.04	72.40	72.41
C-4	68.90	70.21	70.73
C-5	72.72	70.69	72.66
C-6	61.58	62.87	62.05
C-1′	78.83	78.65	78.52
C-2′	103.72	103.40	103.49
C-3′	75.52	75.54	75.58
C-4′	74.86	74.67	74.71
C-5′	62.76	63.10	63.29
C-6′	63.82	64.02	64.12
C-$COCH_3$	169.82–170.96	169.50–170.72	170.23–170.98
C-$COCH_3$	20.36–20.71	20.69–20.81	20.62–20.72

[a] The unit of chemical shift is ppm.

1,3,4,6-Tetra-O-acetyl-β-D-fructofuranosyl 2,3-di-O-acetyl-α-D-glucopyranoside (**4**).—Compound **4** (1.1 g, syrup, 25%) had $[\alpha]_D^{25}$ +57.5° (*c* 1, $CHCl_3$).

1,3,4,6-Tetra-O-acetyl-β-D-fructofuranosyl 2,6-di-O-acetyl-α-D-glucopyranoside (**5**).—Compound **5** (0.306 g, syrup, 7%) had $[\alpha]_D^{25}$ +40.0° (*c* 2, $CHCl_3$).

1,3,4,6-Tetra-O-acetyl-β-D-fructofuranosyl 2-O-acetyl-α-D-glucopyranoside (**6**).—Compound **6** (1.06 g, syrup, 26%) had $[\alpha]_D^{25}$ +47.5° (*c* 1, $CHCl_3$).

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