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Preliminary communication

Glucosylation of some furanosides with 6′-chloro-6′-deoxysucrose and immobilized *Protaminobacter rubrum*

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Protaminobacter rubrum (CBS No. 57477, identical with *P. ruber* which was found by Weidenhagen and Lorenz[1] in 1957) belongs to the *Pseudomonadaceae* family and can convert sucrose (**1**) into 6-*O*-α-D-glucopyranosyl-D-fructose (isomaltulose or palatinose) very effectively. In this conversion, the intermolecular α-D-glucopyranosyl transfer was suggested by the observation that the D-fructose released by the formation of a D-glucosylenzyme was exchanged with added D-[^{14}C]fructose to give [^{14}C]isomaltulose[2]. In addition to D-fructose, monosaccharides such as D-arabinose can serve as acceptor of the D-glucopyranosyl residue. However, the yield[3] of disaccharide formed, 5-*O*-α-D-glucopyranosyl-D-arabinose was only 6%. We improved the efficiency of this transglucosylation substantially by use of 6′-chloro-6′-deoxysucrose (**2**) as donor and methyl β-D-arabinofuranoside (**3**) as acceptor to give methyl 5-*O*-α-D-glucopyranosyl-β-D-arabinofuranoside (**4**) in 72% yield.

Several other pentofuranosides (**5**, **7**, **9**, **11**, and **13**) and a furan derivative (**16**) were glucosylated under the same conditions as follows: To a solution of the donor **2** (1.0 mmol) and an acceptor (1.4 mmol) in 20 mM calcium propionate buffer (pH 5.5) was added the immobilized whole cell of *P. rubrum* (35 mg/1 mmol donor), and the mixture was stirred at 25° for 24 h. Immobilization was done by encapsulation with calcium alginate gel, followed by treatment with aziridine and glutaraldehyde[4]*.

The yields shown in Table I indicate the ratio of donor that was transfered to acceptor, and have only a comparative meaning. It is noteworthy that, in the case of 2-deoxy- (**11**) and 3-deoxy-pentofuranosides (**13**), the secondary hydroxyl groups were also glucosylated, whereas the other pentofuranosides gave 5-*O*-glucosylated disaccharides. The position of the α-D-glucosidic linkage was elucidated by ^{13}C-n.m.r. glucosylation shift (only

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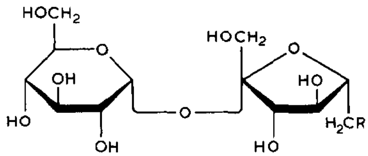
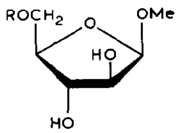
*A cell suspension of *P. rubrum* was mixed with 2% aqueous sodium alginate and extruded into 0.15M calcium chloride solution. The resulting granules were collected by filtration, washed, and treated with 2% aqueous polyaziridine (pH 5.5, adjusted with hydrochloric acid). followed by 0.5% aqueous, glutaraldehyde.



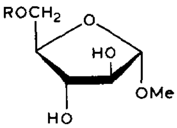
1 R = OH

2 R = Cl



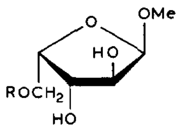
3 R = H

4 R = α-D-Glc*p*



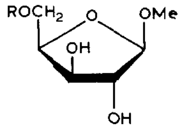
5 R = H

6 R = α-D-Glc*p*



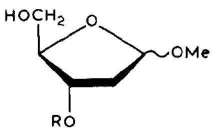
7 R = H

8 R = α-D-Glc*p*



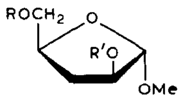
9 R = H

10 R = α-D-Glc*p*



11 R = H (α : β = 1 : 2)

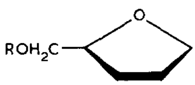
12 R = α-D-Glc*p* (only β)



13 R = R′ = H

14 R = α-D-Glc*p*, R′ = H

15 R = H, R′ = α-D-Glc*p*



16 R = H

17 R = α-D-Glc*p*

α-D-Glc*p* = α-D-glucopyranosyl

TABLE I

TRANSGLUCOSYLATION OF FURANOSIDES AND FURAN DERIVATIVE

Acceptors	*Products*	*Yields (%)*	*Transfer ratios[a] (%)*
3	4	72	100
5	6	11	42
7	8	38	100
9	10	17	42
11	12	35	71
13	14 + 15	15[b]	63
16	17	43	81

[a] Transfer ratio = $\frac{[\text{Product}]}{[\text{Product}] + [\text{Glucose}]} \times 100$, where [Product] and [Glucose] indicate molar proportions of glucosylated product and D-glucose, respectively. [b] 14:15 = 2:1.

TABLE II

PARTIAL DATA FOR ^{13}C-N.M.R. CHEMICAL SHIFTS[a] (δ) OF GLUCOSYLATED PRODUCTS

Products	*Furanose residue*					D-*Glucosyl group*
	C-1	*C-2*	*C-3*	*C-4*	*C-5*	*C-1*
4	103.65	77.48	76.18	81.32 (83.22)	70.52 (64.32)	99.64[b]
6	109.61	81.87	77.15	83.71 (85.06)	68.11 (62.42)	99.75
8	103.37	77.55	76.04	78.19 (79.46)	68.18 (61.69)	99.86
10	109.96	80.87	76.09	81.90 (83.56)	68.43 (62.18)	99.61
12	106.63	38.96 (41.58)	78.24 (72.08)	85.61 (86.88)	64.15	99.72
14	110.60	75.50	34.55	78.48 (80.14)	70.62 (64.81)	99.86
15	108.94 (110.45)	82.58 (75.40)	32.36 (34.60)	80.04	64.96	99.17
17[c]	69.06	26.06	28.31	79.07 78.72 (79.91)	71.21 70.48 (64.75)	99.49 99.20

[a]From the signal of Me_4Si for a solution in D_2O. Data of non-glucosylated acceptors are shown in parentheses only for those which reflect the α and β effect of *O*-glycosylation. [b]Data for other carbons: 72.71 (C-2), 74.33 (C-3), 70.87 (C-4), 73.25 (C-5), and 61.82 (C-6). [c]For a solution in CD_3OD-D_2O.

the data for the furanoside and furan residue are shown in Table II). Furthermore, tetrahydrofurfuryl alcohol was also glucosylated effectively.

In addition to the formation of the disaccharide and 6-chloro-6-deoxy-D-fructose, D-glucose was also formed as the result of glucosyl transfer to water, except for 3 and 7. The ratio of transferred D-glucopyranosyl residue to acceptor is also shown,. in Table I, as transfer ratio. This ratio may be considered as a measure of the fitness of the acceptor to the catalytic site.

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