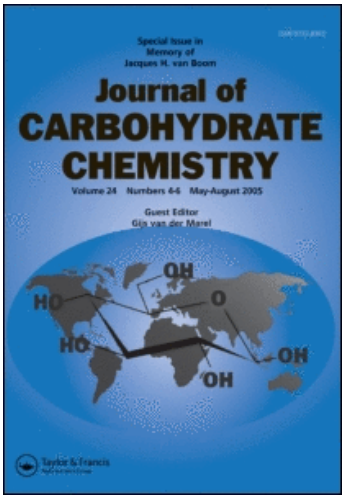

Journal of Carbohydrate Chemistry

Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713617200

Pig Liver Esterase Catalyzed Hydrolysis of Methyl 2,3-Di-*O*-Acetyl-5-Deoxy-α- and β-D-Arabinofuranosides

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To cite this Article Moravcová, Jitka , Hamerník, Ivan , Funková, Gabriela , Čapková, Jindra and Kefurt, Karel(1998) 'Pig Liver Esterase Catalyzed Hydrolysis of Methyl 2,3-Di-*O*-Acetyl-5-Deoxy-α- and β-D-Arabinofuranosides', Journal of Carbohydrate Chemistry, 17: 8, 1191 — 1202
To link to this Article: DOI: 10.1080/07328309808001893
URL: http://dx.doi.org/10.1080/07328309808001893

J. CARBOHYDRATE CHEMISTRY, 17(8), 1191-1202 (1998)

PIG LIVER ESTERASE CATALYZED HYDROLYSIS OF METHYL 2,3-DI-*O*-ACETYL-5-DEOXY-α- AND β-D-ARABINOFURANOSIDES

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Received October 30, 1997 - Final Form June 15, 1998

ABSTRACT

The regioselectivity of a pig liver esterase (PLE) catalyzed hydrolysis of methyl 2,3-di-*O*-acetyl-5-deoxy-α-D-arabinofuranoside (**1**) and methyl 2,3-di-*O*-acetyl-5-deoxy-β-D-arabinofuranoside (**2**) was established by GLC. Diacetate **1** gave exclusively methyl 3-*O*-acetyl-5-deoxy-α-D-arabinofuranoside while diacetate **2** produced both methyl 2-*O*-acetyl-5-deoxy-β-D-arabinofuranoside and methyl 3-*O*-acetyl-5-deoxy-β-D-arabinofuranoside which were resistant to subsequent hydrolysis. The Michaelis constants and maximal velocities were determined for **1** and **2**. The first-order rate constants were computed for **1**, **2**, and all corresponding monoacetates. The results were evaluated on the basis of a Jones's active-site model for PLE and the additional criteria valid for acetyl esters of pentofuranosides were proposed.

INTRODUCTION

In the chemistry of carbohydrates, the introduction or removal of protecting groups is one of the most frequent synthetic transformations. A given hydroxyl group has

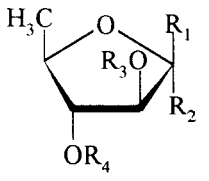
to be protected or unprotected under mild conditions and with a high yield. The use of an acyl group, namely the acetyl group, has been widely accepted in this regard. For the manipulations of ester protecting groups, several enzymic methods have been utilized according to their chemoselectivity, regioselectivity or stereoselectivity.[1] Pig liver esterase (PLE, E. C. 3.1.1.1.), a serine hydrolase, seems to be an enzyme of the first choice because the stereochemistry of its active site as well as the rules controlling the selectivity have been well-documented by a Jones's active-site model.[2]

In a recent paper,[3] we reported the kinetic evaluation of a PLE catalyzed hydrolysis of methyl 2,3-di-*O*-acetyl-5-deoxy-α and β-D-xylofuranosides involving the corresponding mono-*O*-acetyl derivatives. The regioselectivity observed[3] was in full agreement with that published previously for the hydrolysis of (±)-*trans*-cyclopentan-1,2-diol diacetates[4] and methyl 2,3-di-*O*-acetyl-α- and β-D-threofuranosides.[5] The results were interpreted by the Jones's active-site model originally proposed[2] for the prochiral or racemic methyl diesters. Some attempts were made to define the regularities governing the regioselectivity of the hydrolysis of acetate esters derived from cyclic chiral diols.[3]

At present, we decided to extend our study to another model substrate having also two *trans*-oriented vicinal *O*-acetyl groups bound to a flexible furanose ring.

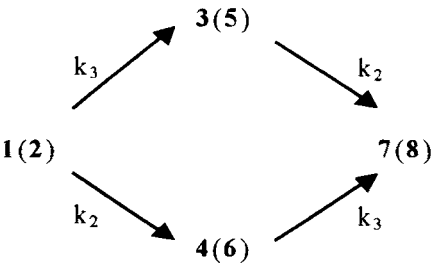
RESULTS AND DISCUSSION

Methyl 2,3-di-*O*-acetyl-5-deoxy-α-D-arabinofuranoside (**1**)[6] and methyl 2,3-di-*O*-acetyl-5-deoxy-β-D-arabinofuranoside (**2**)[6] and the corresponding monoacetates **3** – **6** were isolated from an acetylation mixture of diols **7** and **8**,[6] respectively. Acetylation was performed with a 7-fold molar excess of acetic anhydride in pyridine under kinetic control. An optimized reaction time giving the highest yield of monoacetates was established by GLC. After 60 min, the monoacetates **3** – **6** were obtained in moderate yields ranging from 15 to 30 %. The all new compounds **3** – **6** were identified in the usual manner (optical rotation, MS, ^{1}H and ^{13}C NMR, see Experimental).



	R_1	R_2	R_3	R_4		R_1	R_2	R_3	R_4
1	H	OCH_3	Ac	Ac	**5**	OCH_3	H	Ac	H
2	OCH_3	H	Ac	Ac	**6**	OCH_3	H	H	Ac
3	H	OCH_3	Ac	H	**7**	H	OCH_3	H	H
4	H	OCH_3	H	Ac	**8**	OCH_3	H	H	H

The kinetic scheme of PLE catalyzed hydrolysis of the title compounds **1** and **2** consists of four reactions, each of which is characterized by a rate constant as outlined in the Scheme. The magnitude of the individual rate constants can be influenced by the stability of an appropriate enzyme-substrate (ES) complex and the regioselectivity is determined by the ratio of corresponding rate constants, e.g., k_2/k_3, k_3'/k_2, and k_2'/k_3. The kinetics of PLE hydrolysis of both **1** and **2** were checked by GLC (Figures 1 and 2) and the individual first-order rate constants were calculated (Table 1). In the case of the α-diacetate **1**, the ester group at position 2 is hydrolyzed exclusively to give 3-*O*-acetate **4** in almost quantitative yield. The regioselectivity of the PLE hydrolysis of **1** is clearly controlled by the rate constants $k_2 >> k_3$, $k_3' << k_2$, and $k_2' >> k_3$ (Table 1). In contrast, the PLE hydrolysis of β-diacetate **2** produces both monoacetates **5** and **6** ($k_2 \cong k_3$) which are accumulated during the reaction because the rate of their subsequent hydrolysis substantially decreases ($k_3' << k_2$, $k_2' << k_3$). This is precisely the regioselectivity found for methyl 2,3-di-*O*-acetyl-5-deoxy-α- and β-D-xylofuranosides[3] as well as for methyl 2,3-di-*O*-acetyl-α- and β-D-threofuranosides[5] if the glycosides with the same relative configuration at C-1 and C-2 are compared.



Scheme

In addition, a Lineweaver-Burk plot was used to calculate both Michaelis constant (K_M, $moldm^{-3}$) and maximal velocity (V_{max}, $moldm^{-3}s^{-1}$) for the PLE hydrolysis of the starting diacetates **1** and **2** (Figure 3): **1**, $K_M = 1.43 \times 10^{-2}$, $V_{max} = 7.03 \times 10^{-5}$; **2**, $K_M = 2.02 \times 10^{-3}$, $V_{max} = 1.10 \times 10^{-5}$. In comparison with the K_M found[3] for methyl 2,3-di-*O*-acetyl-5-deoxy-α- and β-D-xylofuranosides, these lower K_M values suggest a

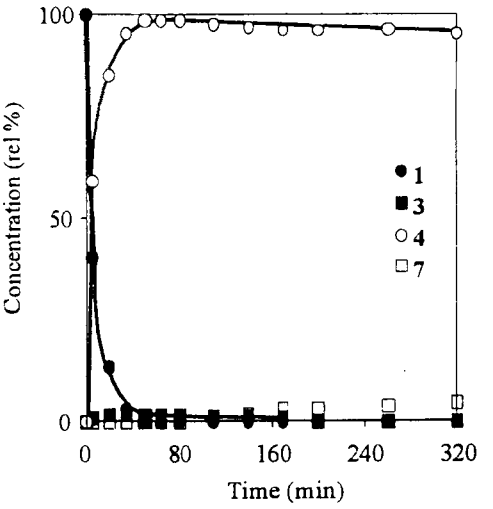


Figure 1. Enzymic hydrolysis of **1**

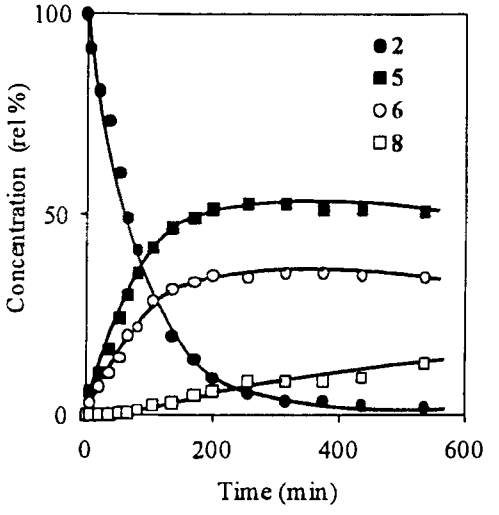


Figure 2. Enzymic hydrolysis of **2**

Table 1. The first-order rate constants for the PLE hydrolysis of acetates **1** and **2**

Substrate	10^4 k (s^{-1}) (Correlation coefficient)				
	$k_2 + k_3$	k_2[a]	k_3[a]	k_2'	k_3'
1	15.53	15.30	0.23	>20[b]	0.015
	(0.9859)	(-)	(-)	(-)	(0.9717)
2	1.98	0.77	1.21	0.02	0.03
	(0.9977)	(-)	(-)	(0.9784)	(0.9319)

a. according to the initial velocities of the formation of individual monoacetates
b. hydrolysis carried out with a half time about 5 min

stronger binding of **1** or **2** into ES complex. Thus, the acetates of 5-deoxy-D-pentofuranosides with the *arabino* configuration represent better substrates for PLE than those having the *xylo* configuration.

When considering possible effects influencing the stability of the ES complexes, it is useful to evaluate the acetates investigated thus far (Table 2) using a generally applicable active site model[2] for PLE. Thus, both ester groups of the β-*arabino* (**2**) and the α-*arabino* (**1**) diacetates are hydrolyzed faster then those of α-*xylo* and β-*xylo* diacetates, respectively ($k_{3arabino}/k_{3xylo}$, $k_{2arabino}/k_{2xylo}$, Table 2).

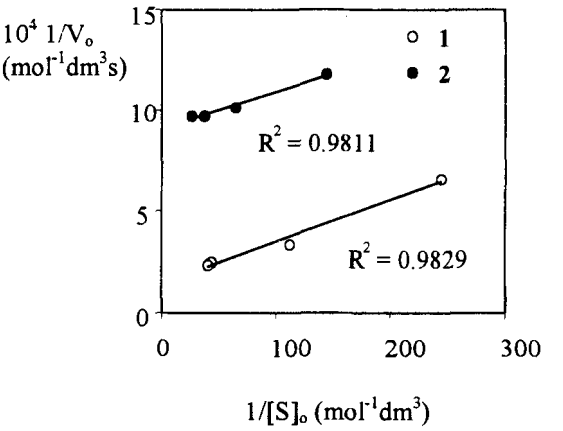


Figure 3. The double-reciprocal plot for the hydrolysis of **1** and **2**

According to the rules,[2,7] the corresponding ES complexes for the hydrolysis of **1** and **2** are characterized by location of the unhydrolyzed acetyl group in the P_F binding site

Table 2. Ratios of the rate constants of the PLE hydrolyses of methyl 2,3-di-*O*-acetyl-5-deoxy-D-pentofuranosides.

Configuration of diacetates	Ratio				
	$k_{3arabino}/k_{3xylo}$	$k_{2arabino}/k_{2xylo}$	k_3/k_2	k_3'/k_2	k_2'/k_3
β-*arabino* (**2**)	2.2	2.6	1.8	0.5	4.7
α-*xylo*[3]			1.6	0.04	0.01
α-*arabino* (**1**)	25	11	<0.01	0.03	97
β-*xylo*[3]			<0.01	0.001	67

(Figure 4). Evidently, this orientation of the vicinal *O*-acetyl groups is energetically favored over that of *xylo* diacetates in which the unhydrolyzed ester group is placed in the P_B zone.[3] Furthermore, this conclusion reveals a lower reaction rate for PLE hydrolysis of methyl 2,3-di-*O*-acetyl-5-deoxy-D-xylofuranosides in comparison with methyl 2,3-di-*O*-acetyl-D-threofuranosides, which has been previously attributed to an effect of the C-4 methyl group.[3] For the substrates displaying a *cis*-relationship between C-1 and C-2 atoms (β-*arabino*, α-*xylo*, Table 2), two essentially equivalent binding modes may be possible. The ES complex of the hydrolysis of 2-*O*-acetyl moiety in **2** (Figure 4c) is stabilized by a hydrogen bond between the methoxyl group and the P_B site and similarly the ES complex for the reaction of 3-*O*-acetyl group (Figure 4d) reflects a hydrophobic interaction into H_L pocket. In contrast, the ES complexes of diacetates with *trans* configuration of C-1 and C-2 (α-*arabino*, β-*xylo*, Table 2) leading to the hydrolysis of 3-*O*-acetyl group are precluded since no additional hydrophobic or polar interaction is involved (Figure 4b).

The important difference between the corresponding monoacetates with *xylo* and *arabino* configurations is the resistance of **5** and **6** to further hydrolysis, which results in their accumulation in a reaction mixture (Figure 2, Table 2). This corresponds to the

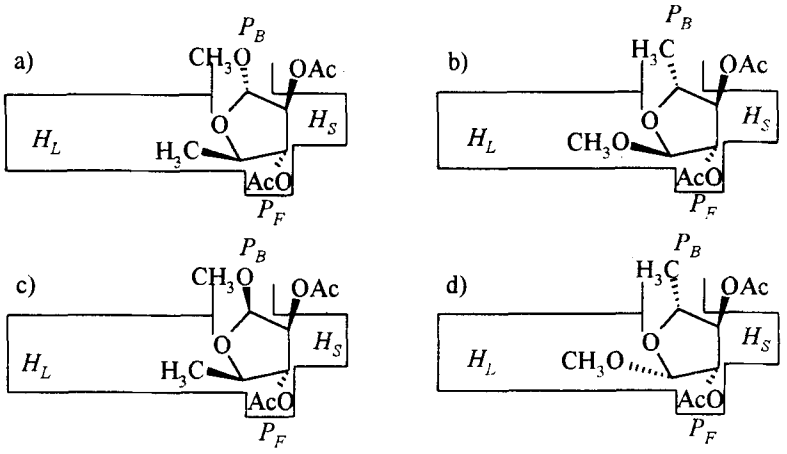


Figure 4. The ES complexes for the hydrolysis of **1** at positions 2 (a) and 3 (b). The ES complexes for the hydrolysis of **2** at positions 2 (c) and 3 (d).

limitation, noted previously,[3] of the inability of the P_F zone to accept a polar hydroxyl group if no additional interaction stabilizes an ES complex.

Finally, the proposed ES complex for the PLE hydrolysis of 2-*O*-acetate **3** illustrates well the additive character of the antagonistic interactions (Figure 5). In this case, the rate of the PLE hydrolysis is high (Table 1) although the binding mode requires the disfavored orientation of the hydroxyl group into the P_F pocket. A strong hydrogen bond between the methoxyl group and the P_B site in the **3**-PLE complex plays a decisive role and thus the hydrolysis of **3** is accelerated in contrast to the monoacetates **5** and **6**.

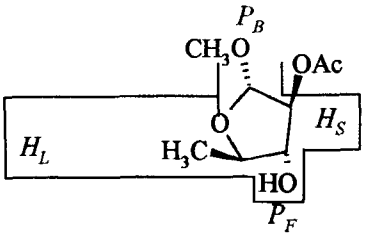


Figure 5. The ES complex for the hydrolysis of **3**

The PLE catalyzed hydrolysis of the title compounds **1** and **2** can be very useful on a preparative scale, the yields of monoacetates **5**, **6** and mainly **4** being substantially higher then those attained through partial acetylation.

CONCLUSIONS

The results of the PLE catalyzed hydrolyses of diacetates of *trans*-cyclopentanediols,[4] methyl threofuranosides,[5] methyl 5-deoxyxylofuranosides,[3] and methyl 5-deoxyarabinofuranosides (this work) can extent the applicability of a Jones's active site model of PLE.[2] Moreover, they imply other important guidelines:

a) The orientation of an unhydrolyzed acetyl group into the P_B site is allowed but its location in the P_F binding site is still energetically favored.

b) The orientations themselves, while allowed according to a), are not sufficient for the hydrolysis. Other interactions with hydrophobic or polar binding site of the enzyme seem to be essential.

c) The most important requirement is the binding of a hydrophobic portion of the substrate into the H_L pocket. Interestingly, the relatively polar methoxyl group can be accommodated in the H_L site as well.

d) The formation of a hydrogen bond with the P_B site stabilizes the ES complex as originally proposed.[2] In this respect, the methoxyl group is preferred over a hydroxyl group.

EXPERIMENTAL

General procedures. Optical rotations were measured on a JASCO Model DIP-370 polarimeter. Melting points were determined with a Kofler hot block and are uncorrected. NMR data were extracted from spectra measured in $CDCl_3$ solutions (Me_4Si as an internal standard) at 25 °C with a BRUKER AM 400 spectrometer (^1H, 400 MHz; ^{13}C, 100.62 MHz). ^{13}C assignments were made using a HETCOR experiment and ^1H NMR shifts were obtained by first order analysis of spectra using a COSY experiment and from selective homodecoupling. Mass spectra were recorded on a JEOL DX 303 instrument using an EI technique at 70 eV. The enzyme catalyzed hydrolyses were carried out under nitrogen in a pH-stat RTS 822 (Radiometer, Denmark) using thermostated vessels with a magnetic stirrer. Porcine live esterase (PLE, 31.4 U/mg of

protein, based on ethyl acetate as a substrate) was purchased from Sigma (USA) as a suspension in 3.2 M $(NH_4)_2SO_4$, containing 8 mg of protein per mL. Column chromatography was performed on Silica Gel Lachema (100-160 μm, Czech Republic), and TLC on Silica Gel according to Stahl (10-40 μm, Merck, Germany): system A, benzene-acetone 8:1; system B, benzene-acetone 1:1. The spots on TLC were detected by spraying with 1% $Ce(SO_4)_2$ in 10% H_2SO_4 and subsequent mineralization. Solutions were concentrated under reduced pressure with a bath temperature below 40 °C.

GLC. Analyses were performed with a Hewlett-Packard 5890 A instrument equipped with a flame-ionization detector. A fused silica capillary column (50m x 0.31mm I.D.) with chemically bonded phenyl methyl silicone (5 %, film thickness 0.5 μ m) was used with nitrogen as a carrier gas at a flow rate 1.8 mL/min (split 1:50). Temperature: 100 °C (5 min), 4 °C/min up to 152 °C, 60 °C/min up to 180 °C, 180 °C (2 min); detector, 230 °C; injector, 200 °C. The following retention times (in min) were obtained: **1**, 15.24; **2**, 15.67; **3**, 10.26; **4**, 10.87; **5**, 11.97; **6**, 11.56; **7**, 8.32; **8**, 6.80.

The Kinetics of the Enzyme Catalyzed Reaction. To a 0.035 M solution (2 mL) of each substrate **1** - **6** in 0.5 M KCl at 25 °C, after adjustment of the pH to 8.0 and stirring for 10 min, PLE (10 μL of original concentrate) was added. By titration with 0.101 M NaOH the pH of solution was kept at 8.0 and aliquots of the reaction mixture (100 μL) were withdrawn during several hours. The reaction was stopped by addition of toluene (100 μL), the mixture was concentrated to dryness, and then diluted with methanol (100 μL). The supernatant was directly analyzed by GLC. The first-order rate constant was computed as a slope of a linear dependence between the logarithm of the molar concentration of the starting compound and reaction time. The reported value of each rate constant is an average of two determinations.

The Determination of K_M and V_{max}. To a solution of each substrate **1** and **2** (0.004 - 0.08 mmol) in 0.5 M KCl (2 mL) at 25 °C and pH 8.0 was added a suspension of PLE (10 μL of original concentrate). An initial time-dependence of consumption of 0.101 M NaOH necessary to maintain pH 8.0 was measured during 10 min. The initial velocity was then calculated as a slope of linear dependence of molar concentration of originating CH_3COOH and reaction time during first minute of reaction.

Table 3. ^{1}H and ^{13}C NMR data for the compounds **3** – **6**

	Compound			
	3	**4**	**5**	**6**
	Chemical shift: δ (ppm)			
H-1	4.92 bs	4.86 s	4.98 d	4.86 d
H-2	4.79 dd	4.08 dd	4.82 dd	4.88 dd
H-3	3.67 dd	4.33 dd	4.15 dd	4.23 dd
H-4	4.14 - 4.07 m	4.22 - 4.16 m	4.04 - 3.97 m	4.03 - 3.98 m
H-5	1.36 d	1.40 d	1.40 d	1.41 d
H-O	3.32 bs	3.20 bs	2.90 bs	2.85 bs
CH_3CO	2.12 s	2.13 s	2.17 s	2.11 s
CH_3	3.39 s	3.38 s	3.40 s	3.47 s
C-1	106.03	108.46	101.64	102.76
C-2	86.39	87.06	81.64	84.67
C-3	81.92	82.53	79.82	77.61
C-4	79.37	75.83	79.23	78.69
C-5	18.41	18.31	21.84	22.11
CH_3CO	20.81	20.77	21.49	21.52
CO	171.58	172.47	172.46	171.76
CH_3O	54.84	54.91	55.74	55.69
	Coupling constants: $J_{H,H}$ (Hz)			
1,2	1.0	0.9	4.5	4.6
2,3	3.1	3.3	7.4	6.2
3,4	6.3	7.6	6.2	4.9
4,5	6.3	6.2	6.4	6.6

Methyl 2-*O*-acetyl-5-deoxy-α-D-arabinofuranoside (3). Methyl 3-*O*-acetyl-5-deoxy-α-D-arabinofuranoside (4). Diol **7** (311 mg, 2.1 mmol) was acetylated with acetic anhydride (1.4 mL, 15.2 mmol) in pyridine (12 mL) at room temperature for 60 min. The excess of acetic anhydride was decomposed by water (2 mL) and the reaction mixture was concentrated to dryness. The residue (360 mg) was separated on silica gel (150 g, system A) to give the diacetate **1** (83 mg, 17 %, R_F 0.61, system A), followed by a monoacetate with R_F 0.31 (system A) which was identified as 2-*O*-acetate **3** (120 mg, 30 %), syrup, $[\alpha]_D^{20}$ +121 (*c* 1, $CHCl_3$). MS and NMR data are given in Tables 3 and 4, respectively.

Anal. Calcd for $C_8H_{14}O_5$: C, 50.53; H, 7.37. Found: C, 50.60; H, 7.41.

Table 4. MS spectra data of compounds **3** – **6**

	Relative abundance								
m/z	**3**	**4**	**5**	**6**	*m/z*	**3**	**4**	**5**	**6**
159	2.5	0.9	2.1	1.7	71	12.3	20.3	10.1	45.7
130	0.8	1.3	1.3	3.4	70	25.6	41.3	36.5	94.7
118	1.3	-	-	-	69	7.3	10.3	6.4	22.8
115	-	-	1.3	0.8	68	5.2	2.4	2.0	2.5
103	-	0.6	0.6	0.8	62	-	0.9	0.8	2.1
100	-	-	0.3	-	61	23.2	35.4	27.1	93.0
99	7.5	3.1	6.1	6.3	60	2.5	4.1	2.0	7.6
98	1.4	0.4	0.5	-	59	7.7	6.8	6.7	14.4
88	5.4	3.3	5.4	7.6	58	25.2	6.5	8.2	7.6
87	40.1	10.5	20.7	18.6	57	19.5	6.5	10.4	10.6
86	17.9	4.1	5.6	3.8	56	1.8	0.4	1.0	-
85	8.9	5.8	4.1	2.0	55	3.9	2.6	2.9	5.1
75	2.1	3.9	1.0	5.9	54	2.7	1.1	1.3	1.7
74	3.9	2.6	2.1	4.2	53	1.4	2.4	1.2	5.1
73	6.6	5.0	5.8	11.0	45	7.3	5.9	4.9	11.0
72	1.5	1.7	1.0	3.4	43	100	100	100	100

Further elution afforded 3-*O*-acetate **4** (60 mg, 15 %, R_F 0.25, system A), syrup, $[\alpha]_D^{22}$ +104 (*c* 0.5, $CHCl_3$). MS and NMR data are given in Tables 3 and 4, respectively.

Anal. Calcd for $C_8H_{14}O_5$: C, 50.53; H, 7.37. Found: C, 50.63; H, 7.48.

The unreacted diol **7** (90 mg, R_F 0.06, system A) was eluted with system B.

Methyl 2-*O*-acetyl-5-deoxy-β-D-arabinofuranoside (5). Methyl 3-*O*-acetyl-5-deoxy-β-D-arabinofuranoside (6). Diol **8** (400 mg, 2.7 mmol) was acetylated with acetic anhydride (1.8 mL, 20.0 mmol) in pyridine (16 mL) at room temperature for 60 min. The mixture was then decomposed by water (3 mL), concentrated to dryness and the residue (600 mg) was separated on silica gel (200 g, system A). Diacetate **2** (160 mg,

26 %, R_F 0.67, system A) was obtained first, followed by 3-*O*-acetate **6** (143 mg, 28 %, R_F 0.48, system A), white solid, mp 73 – 74 °C (diethyl ether - petroleum ether), $[\alpha]_D^{20}$ -109 (*c* 0.5, $CHCl_3$). MS and NMR data are given in Tables 3 and 4, respectively.

Anal. Calcd for $C_8H_{14}O_5$: C, 50.53; H, 7.37. Found: C, 50.65; H, 7.42.

Further elution gave 2-*O*-acetate **5** (120 mg, 20 %, R_F 0.38, system A) as a syrup, $[\alpha]_D^{20}$ -209 (*c* 0.5, $CHCl_3$). MS and NMR data are given in Tables 3 and 4, respectively.

Anal. Calcd for $C_8H_{14}O_5$: C, 50.53; H, 7.37. Found: C, 50.61; H, 7.48.

The unreacted diol **8** (170 mg) was recovered by elution with system B.

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