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ScienceDirect
Journal of Molecular Catalysis B: Enzymatic 44 (2007) 138–143
JOURNAL OF MOLECULAR CATALYSIS B: ENZYMATIC
www.elsevier.com/locate/molcatb

Deacetylation of furanosides using banana as biocatalyst

Marisa Taverna-Porro [a], Luis E. Iglesias [b], Javier M. Montserrat [a,c,*], Adolfo M. Iribarren [a,b]

[a] *INGEBI (CONICET)-Vuelta de Obligado 2490-(1428), Buenos Aires, Argentina*
[b] *Laboratorio de Biotransformaciones, Universidad Nacional de Quilmes, Roque Sáenz Peña 352-(1876) Bernal, Prov. de Bs. As., Argentina*
[c] *Instituto de Ciencias, Universidad Nacional de Gral. Sarmiento, J. M. Gutierrez 1150, Los Polvorines (B1613GSX), Prov. de Bs. As., Argentina*

Received 12 April 2006; received in revised form 29 August 2006; accepted 2 October 2006
Available online 13 November 2006

Abstract

The deacylation under hydrolytic conditions of methyl 2,3,5-tri-*O*-acetyl-α-D-ribofuranoside, methyl 2,3,5-tri-*O*-acetyl-β-D-ribofuranoside, methyl 2,3,5-tri-*O*-acetyl-α,β-D-arabinofuranosides and alkyl 2,3,5-tri-*O*-acetyl-α,β-xylofuranosides have been studied using banana whole tissue as biocatalyst. Reaction regioselectivity strongly depends on substrate structure. Hydrolysis of methyl 2,3,5-tri-*O*-acetyl-α-D-ribofuranoside afforded methyl 2,3-di-*O*-acetyl-α-D-ribofuranoside in quantitative yield.
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Keywords: Regioselective deacetylation; Furanosides; Hydrolases; Banana; Deprotection

1. Introduction

Selective protection–deprotection reactions are usually inevitable steps in the preparation of complex functionalized organic molecules [1]. This problem is particularly cumbersome in carbohydrate chemistry, as a consequence of the presence of hydroxyl functions of similar reactivity [2]. Regioselective synthesis of oligosaccharides necessitates the initial preparation of carbohydrate units with a precise pattern of protection. In addition, properly protected monosaccharides are required synthons for other synthetic applications, such us the preparation of modified nucleosides, a class of compounds that displays antiviral activity [3].

Although numerous chemical techniques are available to mask or liberate carbohydrate hydroxyl groups [4], the enzymatic methods offer an economic and environmentally friendly alternative, displaying a remarkable regioselectivity [5]. The commercial availability of a numerous set of hydrolytic enzymes has prompted the study of acylation–deacylation reactions, specially in pyranose rings [5]. In the case of enzymatic deacetylation, while in acetylated methyl glycosides the primary ester group was preferably cleaved, usually followed by acetyl migration [6], a non-common pattern of hydrolysis was obtained when peracetylated pyranose monosaccharides were assayed [7].

Furanosic monosaccharides have remained as a less studied subclass of molecules. In this sense, Wong and coworkers [8] developed regioselective acylation and deacylation reactions using isolated *Candida rugosa lipase* (CRL) as biocatalyst, which showed regioselectivity towards the 5-deacetylation of methyl 2,3,5-tri-*O*-acetyl-α,β-D-ribofuranosides and methyl 2,3,5-tri-*O*-acetyl-α-D-arabinofuranoside. More recently, the same conditions were applied to 1,2,3,5-tetra-*O*-acetyl-β-D-ribofuranose [9], obtaining the corresponding 5-deacetylated sugar in 80% yield. Guisan and coworkers [10] studied the immobilisation of CRL, among other lipases, on octyl agarose and agarose derivatized with glutaraldehyde; in this case, a similar regioselectivity was displayed and 1,2,3-tri-*O*-acetyl-β-D-ribofuranose was isolated in 47% yield using the first support. Moreover, Kim and coworkers [11] reported in a recent paper that the 2-acetate group of methyl 2,3,5-tri-*O*-acetyl-α-D-arabinofuranoside was regioselectively hydrolyzed in high yield, using commercial pig liver or *Rhizopus oryzae* esterases.

In addition to isolated enzymes, microbial whole cells provide nowadays a wide repertoire of biocatalysts to carry out selective reactions such as ketoester reduction [12], ring hydroxylations [13] and nucleoside preparation [14]. Hydrolytic applications of microbial whole cells are circumscribed to enantioselective hydrolysis of racemic monoesters using fungus [15] or

* Corresponding author at: Instituto de Ciencias, Universidad Nacional de Gral. Sarmiento, J. M. Gutierrez 1150, Los Polvorines (B1613GSX), Prov. de Bs. As., Argentina. Fax: +54 11 4469 7501.
E-mail addresses: jmontser@ungs.edu.ar, jmont@dna.uba.ar (J.M. Montserrat).

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doi:10.1016/j.molcatb.2006.10.001

yeasts [16]. Alternatively, alcohol resolution by enantioselective acetylation using fungus has also been explored [17].

Related to the use of hydrolases from vegetal origin, the hydrolysis of 1-acetoxy-2-methylcyclohexene, using carrot roots as biocatalyst, has been reported [18]. It is also known that certain fruits display hydrolytic activity [19]. Taking into account these facts, we decided to explore the use of banana as biocatalyst in the hydrolysis of alkyl tri-*O*-acetylribo-, arabino- and xylofuranosides. As far as we know, this is the first report of regioselective hydrolysis using vegetal tissues.

2. Experimental

2.1. General

Ethyl acetate, methanol, petroleum ether, hexane, pyridine and methylene chloride were supplied by J.T. Baker, Sintorgan or Ciccarelli. Pyridine and methylene chloride were dried by heating, under reflux, over calcium hydride and distilled at atmospheric pressure. All other reagents were commercially available (Aldrich) and of the best analytical grade.

TLC was performed on Silicagel 60 F_{254} plates (Merck) and column chromatography was carried out using silicagel Merck 60. For TLC analyses, hexane and ethyl acetate at different proportions were used as the mobile phase and revealed using UV light.

NMR spectra were recorded on a Bruker AC-500 spectrometer in $CDCl_3$, at 500 MHz for ^1H and 125 MHz for ^{13}C using TMS and $CDCl_3$ as internal standards, respectively. Chemical shifts (δ, ppm) of multiplets are measured from their centers.

2.2. General procedure for the preparation of diacetylated products

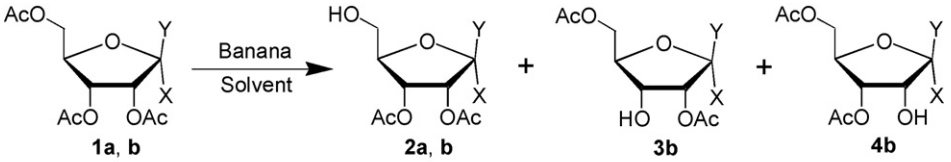
Typically, common Ecuadorian banana (*Musa sapientum*, Cavendish variety, coefficient of ripeness = 1.80 [19]), commercially available at groceries, was cut in cubes (smaller than 1 cm^3) and the small pieces successively washed with 1% $NaClO_{(aq)}$, water, ethanol, water and PBS (Phosphate Buffer Solution, 100 mM, pH 7.0). Banana cubes (10 g) were suspended in PBS, (100 mM, pH 7.0, 10 mL) and the substrate (0.3 mmol) dissolved in DMF or EtOAc depending on the experiment (1 mL of solvent) was added. The mixture was stirred in a reciprocal shaker (37 °C, 200 rpm) until a convenient conversion was reached. Then, the reaction mixture was centrifuged (5 min, 3000 rpm) and the supernatant extracted using EtOAc, dried over anhydrous Na_2SO_4(s), filtered and concentrated in a rotary evaporator. Samples of the crude mixture were withdrawn for NMR analysis and the remaining mixture was purified by silicagel column chromatography using hexane: EtOAc as elution solvents. NMR of the purified and crude mixtures were compared to verify that no acetyl migration took place.

Control experiments performed in absence of banana with the starting materials **1a**, **1b**, **5a** + **5b**, **9a** + **9b** and **10a** + **10b** showed that no hydrolysis occurred.

3. Results and discussion

3.1. Ribosides

Initially, pure methyl 2,3,5-tri-*O*-acetyl-α-D-ribofuranoside (**1a**, Fig. 1), obtained as previously described [20], was hydrolysed using pretreated common Ecuadorian bananas as biocatalyst. Reactions were followed up by TLC until complete disappearance of **1a**. Two experimental conditions were explored; first, a mixture of DMF and PBS pH 7.0 was used as solvent [8]. The ^1H NMR of the crude mixture (Fig. 2A) indicated the presence of a sole product (72% yield, Fig. 1, only one anomeric carbon), in which C-5 hydrogens have experienced a strong up-field displacement, in agreement with the spectroscopic data previously reported for methyl 2,3-di-*O*-acetyl-α-ribofuranoside [8] (**2a**, Fig. 1). When the solvent was changed to a mixture of ethyl acetate (EtOAc): PBS pH 7.0, the reaction conducted again to a only one product in quantitative yield (Fig. 1). The improvement in reaction yield could be attributed to a cleaner liquid–liquid extraction since the DMF/PBS system produced persistent interface emulsions.



1a, b **2a, b** **3b** **4b**

X	Y	Compd.	Solvent	Molar Ratio **2** : **3** : **4**	Conv. %[b]	Yield %[c]	Reaction time/h
OCH_3	H	a	DMF:PBS[a]	1: 0 : 0	100	72	11
OCH_3	H	a	EtOAc:PBS	1 : 0 : 0	100	99	11
H	OCH_3	b	DMF:PBS	1 : 1 : 1	83	43	13
H	OCH3	b	EtOAc:PBS	1 : 1 : 1	85	54	12

[a] PBS: Phosphate Buffer pH=7.
[b] Determined using mass balance of the chromatographically purified products.
[c] After purification on silica gel column except for compound **2b** for which purification was not neccessary.

Fig. 1. Dceacetylation of methyl 2,3,5-tri-*O*-acetyl-α-D-riboside (**1a**) and methyl 2,3,5-tri-*O*-acetyl-β-D-ribofuranoside (**1b**) using banana as biocatalyst.

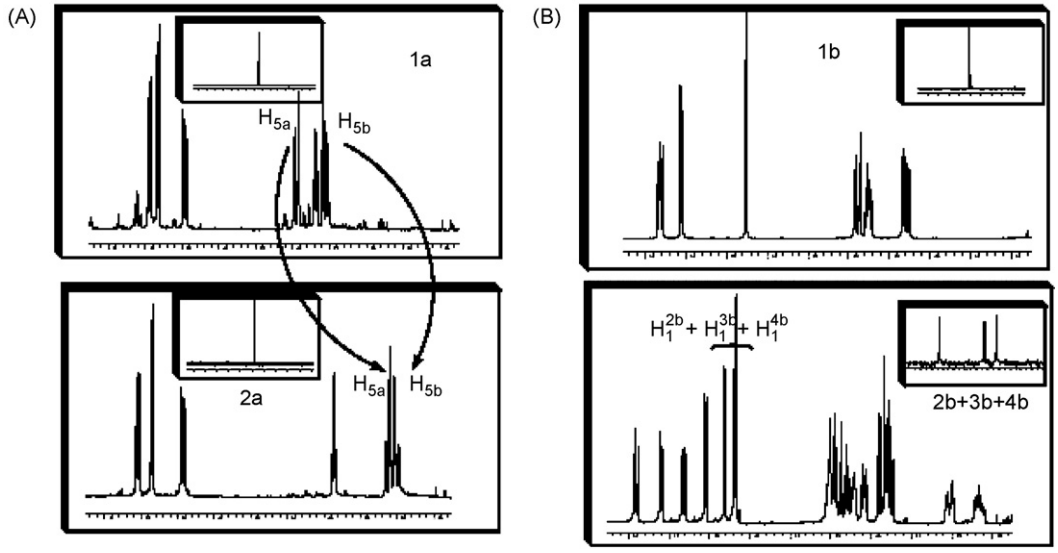


Fig. 2. ^{1}H NMR and ^{13}C NMR data of the starting materials **1a** and **1b** and their corresponding crude reaction mixtures.

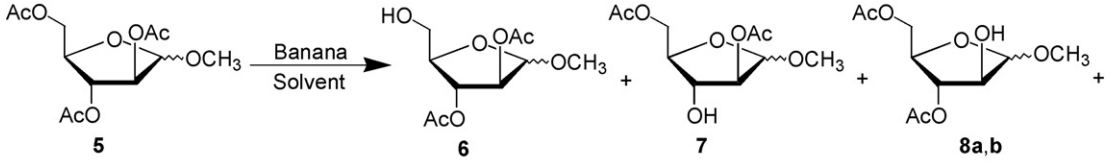
When the reaction was performed using pure methyl 2,3,5-tri-*O*-acetyl-β-D-ribofuranoside (**1b**, Fig. 1) a non-regioselective deprotection was observed. In this case an equimolecular mixture of methyl 2,3-di-*O*-acetyl-β-D-ribofuranoside (**2b**, Fig. 1), methyl 2,5-di-*O*-acetyl-β-D-ribofuranoside (**3b**, Fig. 1) and methyl 3,5-di-*O*-acetyl-β-D-ribofuranoside (**4b**, Fig. 1) was obtained. This result is supported spectroscopically (Fig. 2B) since a set of three different signals were observed by ^{1}H NMR, corresponding to three different H-1 (4.86, 4.87 and 4.92 ppm), which correlate with the three different anomeric signals observed by ^{13}C NMR (105.86, 106.39, 108.42 ppm). The mixture presented a single spot on TLC with the same R_f as compound **2a** indicating the same deacetylation degree.

Although Wong and coworkers [8] reported that **1a** and **1b** exhibited the same regioselective behaviour in the CRL catalysed hydrolysis, the results here obtained suggest that the stereochemistry at the anomeric center could affect the selectivity of the enzymatic deacetylation when using banana as biocatalyst. In line with these results, we recently reported [20] that in enzymatic alcoholysis, *Candida antarctica* B lipase displays a different recognition pattern towards the anomers **1a** and **1b**.

Related to the nature of the biocatalysts displaying hydrolytic activity in banana tissues, protease and lipase activities have been previously reported [19].

3.2. Arabinosides

In order to explore further regioselective hydrolyses, methyl 2,3,5-tri-*O*-acetyl-α,β-D-arabinofuranosides (**5a**,**b**; Fig. 3) were prepared. All ^{1}H NMR signals of **5a**,**b** were assigned by homodecoupling experiments, but it was not possible to chromatographically separate both anomers. The anomeric mixture (α:β, 2:1) was then assayed under the same reaction conditions employed for ribosides (DMF:PBS pH 7, 37 °C), and analysed at different conversion degrees. At 45% conversion (Fig. 3), only diacetylated products **6a**,**b**, **7a**,**b** and **8a**,**b** were obtained



+ Didesacetylated products

Solvent	Molar Ratio **6** : **7** : **8** (**8a** : **8b**)	Conv. %[b]	Monodesacet. yield %[c]	Didesacet. yield %[c]	Reaction time/h
DMF:PBS[a]	2 : 1 : 7 (1 : 1.2)	45	25	0	10
DMF:PBS	2.6 : 2.1 : 5.3 (1 : 0.04)	87	17	45	16
EtOAc:PBS	1 : 1 : 8 (1 : 1.1)	50	27	0	10
EtOAc:PBS	1.7 : 2.8 : 5.6 (1 :0.18)	100	14	64	16

[a] PBS: Phosphate Buffer pH=7.
[b] Determined using mass balance of the chromatographically purified products.
[c] After purification on silica gel column.

Fig. 3. Deacetylation of methyl-2,3,5-tri-*O*-acetyl-α,β-arabinofuranoside (**5a**,**b**) using banana as biocatalyst.

as a mixture in a molar ratio of 2:1:7. The major components were assigned as a mixture of methyl 3,5-di-*O*-acetyl-α,β-D-arabinofuranoside (**8a**:**8b**, 1.2:1). NMR comparison of the crude mixture and the chromatographically isolated fraction suggested that no acetyl migration occurred during purification.

When the reaction reached 87% conversion (Fig. 3), the mixture of **6a**,**b**, **7a**,**b** and **8a**,**b** was isolated in a ratio 2.6:2.1:5.3 (ratio **8a**:**8b**, 0.04:1). In this case a mixture of monoacetylated compounds, not resolved by silica gel column, was also formed in 45% yield. These results contrasted with the performance of riboside **1a**, which could be quantitatively converted into the diacetylated product (**2a**) without traces of further hydrolysis.

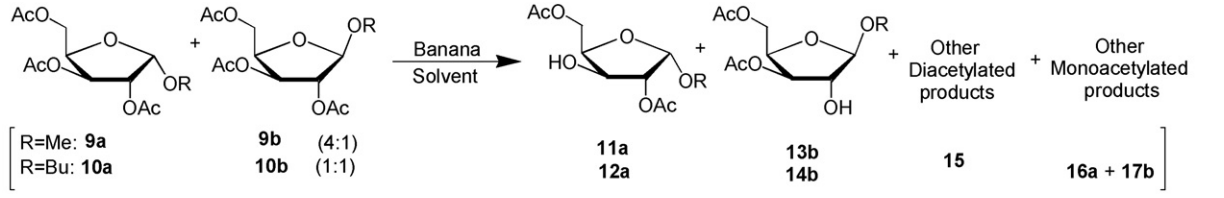
When EtOAc:PBS pH 7 conditions were assayed using **5a**,**b** as starting material, similar results as with DMF:PBS were obtained (Fig. 3). In both cases when the conversion degree was increased the monoacetylated products yields were mainly raised by hydrolysis of **8a**, reflected in the change of **8a** and **8b** ratio (Fig. 3).

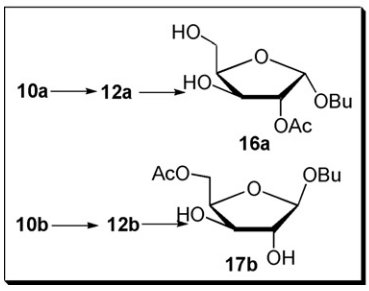
3.3. Xylosides

The hydrolyses of alkyl 2,3,5-tri-*O*-acetyl-α,β-D-xylofuranosides (**9a**,**b** and **10a**,**b**, Fig. 4) have also been explored showing, as in the riboside case, differential regioselectivity depending on C-1 configuration.

When methyl xylosides **9a**,**b** (*α*:*β*, 4:1) were hydrolysed in DMF:PBS (pH 7) using banana as biocatalyst, a mixture containing methyl 2,5-di-*O*-acetyl-α-D-xylofuranoside (**11a**) and methyl 3,5-di-*O*-acetyl-β-D-xylofuranoside (**13b**) was obtained (42% yield at 65% conversion) in a molar ratio of 5.3:3.8 (Fig. 4) along with approximately 10% of other diacetylated products. When the same reaction progressed to a higher conversion degree (86% conversion, Fig. 4), **11a** and **13b** were isolated in lower yields, what could be attributed to subsequent reaction hydrolysis. As **11a** and **13b** were difficult to separate, the hydrolysis was assayed using a more lipophylic substrate. For this purpose butyl 2,3,5-tri-*O*-acetyl-α,β-D-xylofuranosides (**10a**,**b**) were prepared as a 1:1 anomeric mixture. When the reaction was performed with this mixture, 40% of diacetylated fraction mainly composed by **12a** and **14b** (Fig. 4) and 28% of **16a** and **17b** were obtained at 76% conversion (Fig. 4). In the diacetylated fraction a 2.2:3.4 ratio between compounds **12a** and **14b** was observed and a pure fraction of compound **14b** was chromatographically isolated, what allowed confirmation of the deacetylated positions by NMR analysis (Fig. 5). For compound **14b** a clear H2 shift (from 5.60 ppm in compound **10b** to 4.55 ppm in compound **14b**, black circle at Fig. 5) showed that deprotection occurred at this position. For compound **12a** the H3 displacement (from 5.13 ppm in compound **10a**, white circle Fig. 5, to 4.20 ppm in compound **12a**) indicated the hydrolysed acetate. It is interesting to remark that an improvement in the regioselectivity was observed, in comparison to methyl xylosides, as shown by the diminution of other diacetylated compounds (Fig. 4). When the conversion proceeded up to 95%, the yield of monoacetylated products raised to 51% (Fig. 4). By NMR analysis (C1 107.34 and 98.83 ppm, H1 5.21 ppm as a doublet and 4.97 ppm as a singlet) it was possible to establish that the mixture was mainly formed by a 1:1 ratio of *α*:*β* isomers (Fig. 5). Based on homodecoupling analysis of the mixture, the products were assigned as butyl 2-*O*-acetyl-α-D-xylofuranoside (**16a**, Fig. 5) and butyl 5-*O*-acetyl-β-D-xylofuranoside (**17b**, Fig. 5). For compound-**16a** a clear up-field displacement (white circles, Fig. 5) of H3 and H5 (shift of 0.78 and 0.50 ppm, respectively) was observed, while for **17b** the up-field shift (black circle, Fig. 5) was associated to H2 and H3 (shift of 0.94 and 0.84 ppm, respectively).

In order to verify if this hydrolytic procedure could be applied to the removal of benzoyl groups, 3,5-di-*O*-acetyl-1,2-isopropyliden-α-D-xylofuranoside and 3,5-di-*O*-benzoyl-1,2-isopropyliden-α-D-xylofuranoside have been prepared and assayed with banana tissues. Meanwhile the diacetylated xyloside was hydrolysed following a non-regioselective pattern, no evidence of benzoyl removal was detected at least after 48 h of reaction.





Starting material	Conv. %	Diacet. yield %	Monoacet. yield %	11a : 13b : 15	12a: 14b : 15 : 16a+17b[a]
9a+9b	65	42	NM	5.3 : 3.8 : 1.1	-
9a+9b	86	29	NM	6.2 : 2.5 : 1.3	
10b+10b	76	40	28		2.2 : 3.4 : 0.2 : 4.2
10a+10b	95	8	51	-	0.52 :0.84 :0.04 : 8.6

[a] Monoacetylated products as a mixture of α and β anomers.
NM: not measured.

Fig. 4. Hydrolysis of alkyl 2,3,5-tri-*O*-acetyl-α,β-D-xylosides (**9a**,**b**; **10a**,**b**) using banana as biocatalyst.

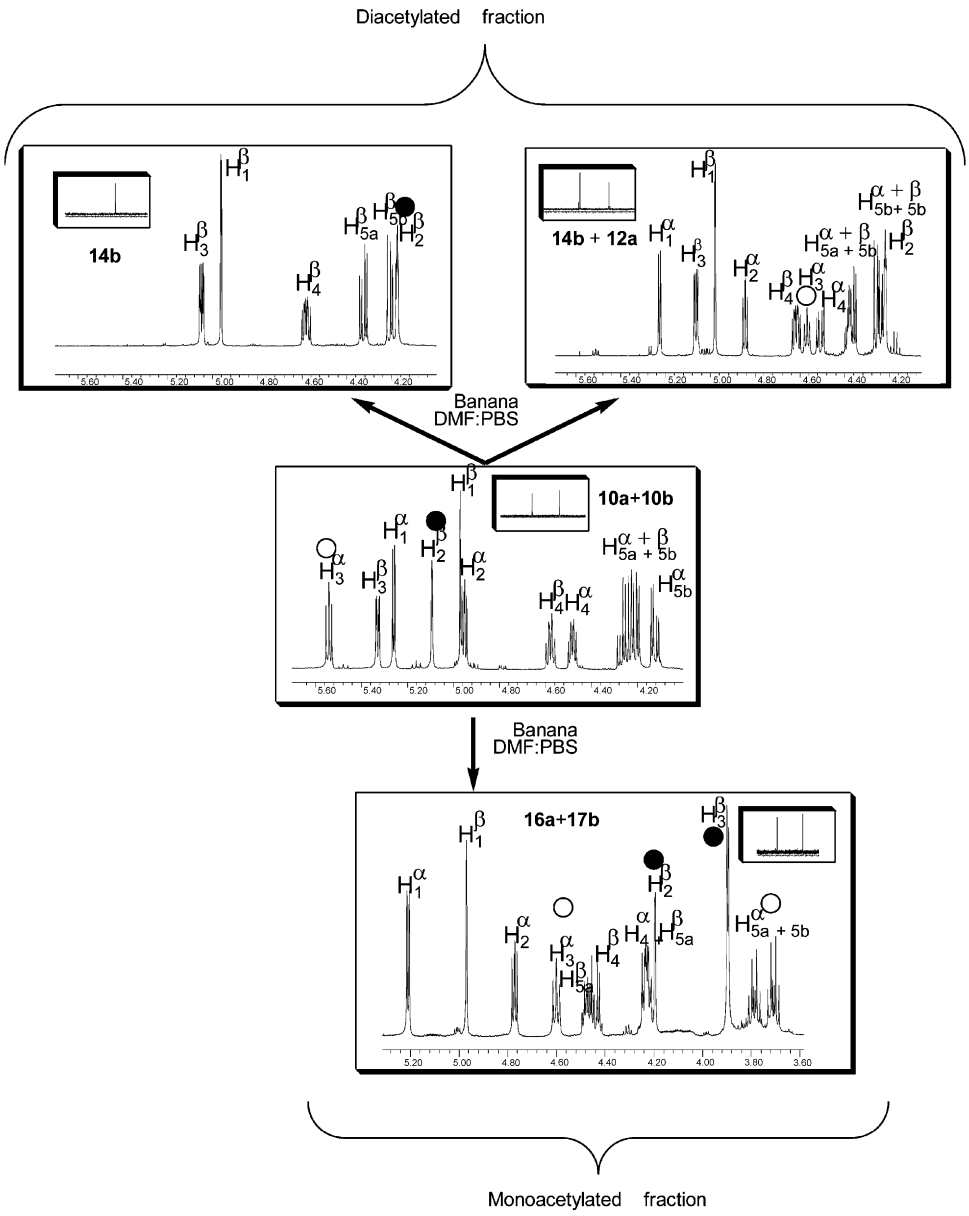


Fig. 5. ^{1}H NMR spectra of products obtained in the hydrolysis of **10a**,**b** using banana as biocatalyst.

4. Conclusions

Regioselective hydrolysis reactions with non-cultured cell plants could be considered as a useful synthetic tool specially when compared with the use of fungus, yeast or bacteria since no biological skills are required. For the studied furanosides, reaction regioselectivity strongly depends on substrate structure. In the case of methyl 2,3,5-tri-*O*-acetyl-α-D-ribofuranoside the reaction was highly regioselective affording quantitatively methyl 2,3-di-*O*-acetyl-α-D-riboside.

Acknowledgments

We thank UNQ, CONICET and SECyT for partial financial support. LEI, JMM and AMI are research members of CONICET.

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