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^{1}H NMR Acetyl Methyl Resonances of Peracetylated Lilioside B: a Tool for the Stereochemical Identification of its Partially Acetylated Derivatives

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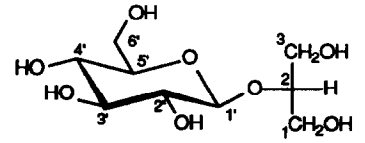
Abstract: Through ^{13}C-^{1}H heteronuclear shift correlation experiments all the acetyl methyl resonances of 1,3-di-*O*-acetyl-2-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)glycerol and of the diastereoisomeric (2R) and (2S)-1-*O*-acetyl-2-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)glycerol have been assigned in the ^{1}H NMR spectra; partially acetylated derivatives of 2-*O*-(β-D-glucopyranosyl)glycerol, lilioside B, can so be easily identified and their configuration established also when mixtures of several derivatives are under investigation.

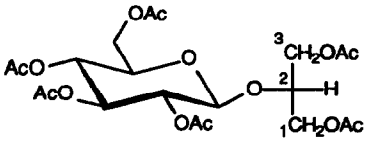
In the course of our studies on the regioselective acylation of sugars mediated by lipases in organic solvents[1] we faced the problem of determining the composition of the reaction mixtures coupled with the problem of the regio and stereochemical identification of the products. In particular, the study of the acetylation of glucosylglycerols, like 2-*O*-(β-D-glucopyranosyl)glycerol, (lilioside B, **1**),[2] represents a particularly intriguing task especially as far as the regio and diastereoselectivity of the esterification reaction is concerned as these compounds have several primary and secondary hydroxyl functions (three and three, respectively, in the case of **1**). The reaction mixtures in such acylation reactions may contain different mono- or multi-acetylated derivatives so that an easy way of detection is requested.

The method of "perdeuterioacetylation", i.e. the acetylation of partially acetylated products with [^{2}H$_6$]acetic anhydride, has been proposed[3] as a procedure able to distinguish the individual acetyl groups with the aid of NMR techniques. This method could be utilized in the stereochemical analysis of our esterification mixtures provided that all the acetyl methyl groups in peracetylated lilioside B are assigned in the ^{1}H NMR spectra.

Here we describe the assignment of all the methyl resonances - including the diastereotopic acetyl methyl groups of the glycerol moiety - in the ^{1}H NMR spectrum of 1,3-di-*O*-acetyl-2-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)glycerol (**2**) performed through ^{13}C-^{1}H heteronuclear shift correlation experiments and comparison with the pentaacetates (2R) and (2S)-1-*O*-acetyl-2-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-glycerol (**3**) and (**4**) and the products of their deuterioacetylation **5** and **6**.



1



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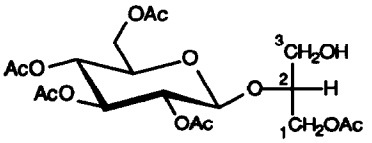

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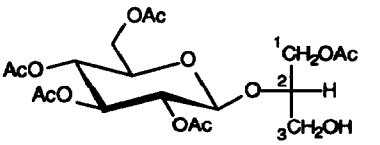
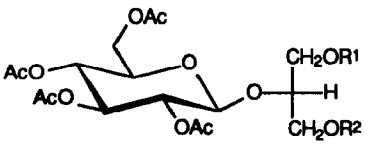
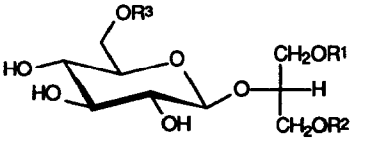


4



5: $R^1 = COCD_3$, $R^2 = Ac$
6: $R^1 = Ac$, $R^2 = COCD_3$



7: $R^1 = R^3 = H$, $R^2 = Ac$
8: $R^1 = Ac$, $R^2 = R^3 = H$
9: $R^1 = R^2 = H$, $R^3 = Ac$
10: $R^1 = H$, $R^2 = R^3 = Ac$
11: $R^1 = R^3 = Ac$, $R^2 = H$
12: $R^1 = R^2 = Ac$, $R^3 = H$

RESULTS AND DISCUSSION

The ^1H NMR spectrum of **2** in $CDCl_3$ at 500 MHz exhibits six well-resolved signals for the acetyl methyl groups (figure 1a) and also the carbonyl carbon region in the ^{13}C NMR spectrum has a good dispersion of the six signals. On the basis of the already assigned[4] methine and methylene signals it is expected an easy acetyl group assignment through a COLOC experiment.[5,6] Actually, two experiments were necessary, in the first the spectrum was acquired under conditions in which the cross-peaks arising from coupling between a carbonyl carbon and a pyranose ring proton appear more intense than those arising from coupling between a carbonyl carbon atom and the methylene protons of the three CH_2OAc groups; in the second the conditions were modified in order to obtain more intense cross-peaks for the latter than for the former correlation. In this way, four in the six signals were easily assigned (table 1), the only doubt being the assignment of the diastereotopic carbonyl carbons and of the acetyl methyls of the glycerol moiety.

To do that, we took into consideration the penta-acetates **3** and **4** whose configuration had been already assigned.[2] First of all new COLOC experiments on the two penta-acetates were performed; once again two distinct experiments were necessary for the assignment of the acetates on the primary and secondary positions, respectively. The assignments of the five signals of the acetyl methyl groups in the ^1H NMR spectra and of the carbonyl signals in the ^{13}C NMR spectra were straightforward and were easily derived from the already assigned[2] glucose and glycerol proton signals.

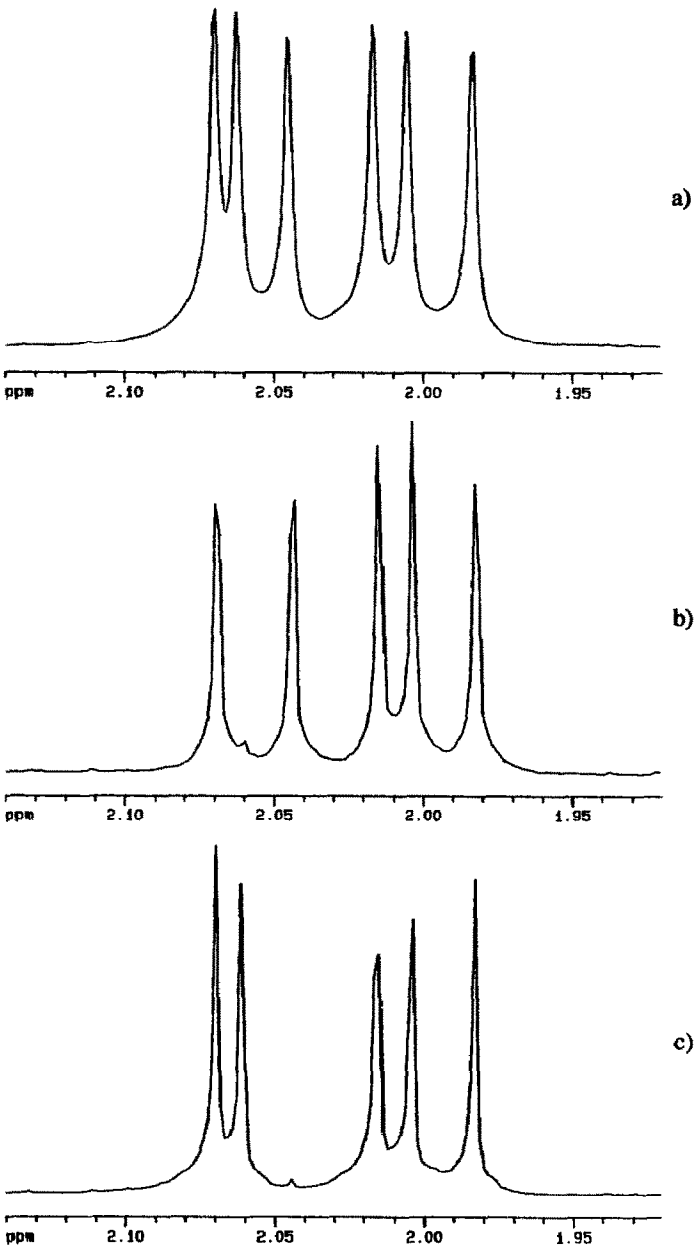


Fig. 1. Comparison of the ^{1}H NMR spectra of compounds a) **2**, b) **5**, and c) **6** in the acetate-resonance region.

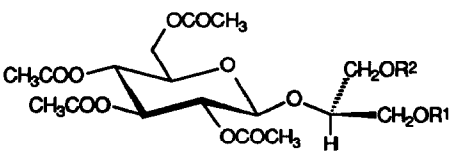
Table 1. Chemical Shifts (δ in ppm from Tetramethylsilane) for the Acetyl Methyl (^{1}H NMR) and Carbonyl (^{13}C NMR) Signals of Compounds **2**, **3**, and **4** in $CDCl_3$.

	compound **2**	compound **3**	compound **4**
1-acetyl methyl	2.04 (pro-R)	2.04	2.04
3-acetyl methyl	2.06 (pro-S)	-	-
2'-acetyl methyl	2.01	2.03	2.02
3'-acetyl methyl	1.98	1.98	1.98
4'-acetyl methyl	2.00	2.00	2.00
6'-acetyl methyl	2.07	2.06	2.06
1-acetyl carbonyl	170.46 (pro-R)	170.80	170.52
3-acetyl carbonyl	170.37 (pro-S)	-	-
2'-acetyl carbonyl	169.06	169.61	169.15
3'-acetyl carbonyl	170.15	170.19	170.13
4'-acetyl carbonyl	169.27	169.37	169.33
6'-acetyl carbonyl	170.52	170.64	170.52

A comparison of the ^{13}C carbonyl chemical shifts in compounds **2-4** in table 1 shows that only the 2'-acetyl carbonyl in the four glucose substituents is sensitive to the substitution pattern on the glycerol moiety, in particular there is a shift of about 0.5 ppm in **3** but not in **4** with respect to **2** due to the conformation assumed by these compounds (figure 2), which shows the spatial proximity of the 2'-acetyl methyl with the CH_2OR^1 group of glycerol which is acetylated in **2** and **4** but not in **3**.

Deuterioacetylation of **3** yielded the penta-acetate-monodeuterioacetate **5**; note that, in spite of the retention of configuration, the priority rules yield the R designation to the starting pentaacetate **3** but the S designation to the monodeuterioacetate product **5**. Similarly, the 2S compound **4** yielded the 2R product **6**. Compounds **5** and **6** differ from **2** only for the isotopic substitution on one of the diastereotopic acetyl groups of the glycerol moiety. Their ^{1}H NMR spectra were in all the respects identical to the spectrum of **2** except for the acetyl methyl resonance region at about 2 ppm (figure 1b,c). In fact compound **5** lacks the signal at 2.06 ppm and compound **6** the signal at 2.04 ppm; accordingly in the spectrum of **2** the signals at 2.04 and 2.06 ppm can be attributed to the pro-R and pro-S acetyl groups, respectively. The assignment of the signals of the corresponding carbonyl carbons in the ^{13}C NMR spectra has been made consequently; so, the signal at 170.46 ppm was due to the pro-R group and the signal at 170.37 ppm to the pro-S one.

With all these assignments made, it is now possible an easy stereochemical analysis of more or less complex mixtures of acetylated derivatives of **1**. The monoacetylated fraction derived from the trans-esterification with vinyl acetate on **1** performed in pyridine in reactions catalyzed by the lipases from



2: $R^1 = R^2 = Ac$
3: $R^1 = H$, $R^2 = Ac$
4: $R^1 = Ac$, $R^2 = H$

Fig. 2. Preferred conformation of compounds **2-4**.

Table 2. Relative Intensities of the Signals of the Acetate Region of the ^1H NMR Spectra Recorded after Perdeuterioacetylation of the Mono- and Di-acetate Fractions from Transesterification on **1** Catalyzed by Lipases from *Candida antarctica* (LCA) and *Pseudomonas cepacia* (LPS).

	relative intensities of the acetyl methyls						composition of the mixtures	
	1	3	2'	3'	4'	6'	**7:8:9**	**10:11:12**
monoacetates (LCA)	100	21	a	a	a	8	78:16:6	-
monoacetates (LPS)	12	100	a	a	a	10	10:82:8	-
diacetates (LCA)	100	42	a	a	a	80	-	62:10:28
diacetates (LPS)	19	100	a	a	a	95	-	7:82:11

[a] About 1% due to the non-deuterated acetic anhydride present in the [$^2H_6$]acetic anhydride.

Candida antarctica and *Pseudomonas cepacia* and whose composition had already been determined[2] was perdeuterioacetylated and in table 2 are reported the intensities of the signals in the acetate region of the ^1H NMR spectra. Mass spectrometric analysis performed under chemical ionization by ammonia assured that these mixtures were composed only by mono-acetate-penta-deuterioacetate derivatives as only the $[M+NH_4]^+$+15 peak was detected. Thus, the individual acetates **7**, **8**, and **9** in the original mixtures could be easily identified together with their relative abundance confirming the results reported in reference 2.

In the above mentioned lipase catalyzed transesterification reactions a certain amount of diacetylated derivatives of lilioside B (**1**) was also obtained when longer reaction times were allowed. These diacetylated fractions were perdeuterioacetylated and mass spectrometric analysis indicated the presence of the only $[M+NH_4]^+$+12 peak. The acetyl methyl region in the ^1H NMR spectra (table 2) allowed to establish that the original mixtures contained only the diacetates **10**, **11**, and **12** in 62:10:28 ratio in the reaction catalyzed by *Candida antarctica* lipase and in 7:82:11 ratio in the reaction catalyzed by *Pseudomonas cepacia* lipase. Thus, the 2R compound **10** was shown the main diacetylated product in the reaction with the former enzyme and the 2S compound **11** in the reaction with the latter enzyme; the product distribution in the second acetylation step of the transesterification reactions reflects that already established in first acetylation step.[2]

In conclusion, in this paper we have assigned the acetyl methyl resonances in the ^1H NMR spectrum of **2**, **3** and **4**; these assignments, coupled with perdeuterioacetylation, are a useful tool for the analysis of mixtures of known and unknown acetyl derivatives of lilioside B (**1**), as evidenced in the above examples.

Acknowledgements

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EXPERIMENTAL

1,3-di-*O*-acetyl-2-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)glycerol (**2**),[4] (2R)-1-*O*-acetyl-2-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)glycerol (**3**)[2] and (2S)-1-*O*-acetyl-2-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)glycerol (**4**)[2] were prepared according to previously published procedures.

Perdeuterioacetylation Procedure. (2S)-1-*O*-[$^2H_3$]acetyl-3-*O*-acetyl-2-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)glycerol (**5**) and (2R)-1-*O*-[$^2H_3$]acetyl-3-*O*-acetyl-2-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)glycerol (**6**) were prepared treating 3 mg of **3** and **4**, respectively, with 0.1 mL of [$^2H_6$]acetic anhydride (99% from Aldrich) and 0.1 mL of pyridine for 3 hours at room temperature. The crude reaction mixtures were dried under vacuum and directly submitted to ^1H NMR analysis. The monoacetate and diacetate fractions from the reactions of **1** and vinyl acetate catalyzed by *Candida antarctica* and *Pseudomonas cepacia* lipase[2] (3 mg) were similarly perdeuterioacetylated using 0.3 mL of [$^2H_6$]acetic anhydride and 0.3 mL of pyridine.

NMR Experiments. NMR spectra were recorded with a Bruker AM-500 spectrometer, equipped with an Aspect 3000 computer, a process controller and an array processor, in deuterochloroform solutions at 303 K. The COLOC spectra[5] were acquired by using a spectral width of 500 Hz in the F_2 dimension, and 2000 Hz in the F_1 dimension. The fixed delay times Δ_1 and Δ_2 were 90 ms and 90 ms, in order to emphasize long-range couplings of the carbonyl carbons to the pyranose-ring protons, or 160 ms and 110 ms to observe the correlations of the carbonyl carbons to the methylene protons of the acetyloxymethyl groups. Each spectrum was collected by using 256 experiments with a spectral size in the time domain of 0.5 K, 112 acquisitions, 1.5 s of relaxation delay and 0.5 s of acquisition time.

Mass Experiments. Mass spectroscopy was performed on a particle beam quadrupolar mass spectrometer Hewlett-Packard HP 5988A (Palo Alto, California) equipped with an interface PB 59980A and a low pressure HPLC HP 1050. The mass spectrometric analysis was performed in Positive Ion Chemical Ionization (PICI) with ammonia as chemical reactant gas at an electron energy of 240 eV and with a source temperature of 250 °C. The particle beam desolvation chamber temperature was 45 °C, the helium pressure was 45 psi and the source pressure 1 torr. Mass spectra were acquired over 50-600 mass unit range at 0.78 scan sec^{-1}. Samples (3 mg) were dissolved in methanol (0.5 mL) and introduced (3 μL) into the mass spectrometer *via* the particle beam LC/MS interface directly connected with the HPLC system, eluting with methanol (0.4 mL/min).

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