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Stereospecific Chemoenzymatic Synthesis of the Deuterated analogues of 2-Phosphoglycerate and Structural analysis by means of NMR Spectroscopy

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Abstract *: A stereospecific chemoenzymatic synthesis of (2-R,3-S)[2,3-^{2}H]-2-phosphoglycerate. and of its (2-R,3-R)[2,3-^{2}H] epimer is presented. The method used involves the preparation of (E)- and (Z)-[3-^{2}H]-phosphoenolpyruvate followed by the addition of water catalyzed with the enolase. The title compounds enable the attribution of the pro-R and pro-S protons of the 2-phosphoglycerate and thus allows us to discuss the conformations for 2-phosphoglycerate. On the basis of the shifts induced by lanthanide salts ($PrCl_3$), which are shown to complex efficently the phosphate function and considering the coupling constants values, a gauche conformation with a dihedral angle H_2-C_2-C_3-H_{3S} near 90° was found to be predominant.*

Introduction

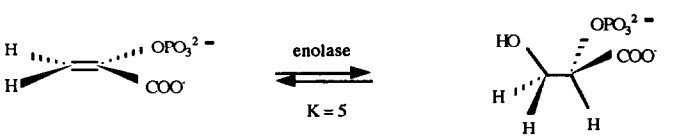
2-Phosphoglycerate is a very important metabolite in glycolysis. Up to now very little structural spectroscopic data has been available for this compound[1] and particularly in ^{1}H NMR spectroscopy the assignment of the two methylenic protons was not proved unambiguously, thus precluding a right conformational analysis for this molecule. Furthermore, the attribution of the pro-R and of the pro-S protons is important for the study of the proton transfers occuring in the fermentation of sugars[2,3,4] since they originate from the 6,6'-protons of the glucose without any exchange with the media.

The aim of this work is to present a methodology allowing the enantioselective synthesis of the deuterated analogues of 2-phosphoglycerate and as a matter of fact to assign the two methylenic protons and to analyse the conformational isomers present for this compound.

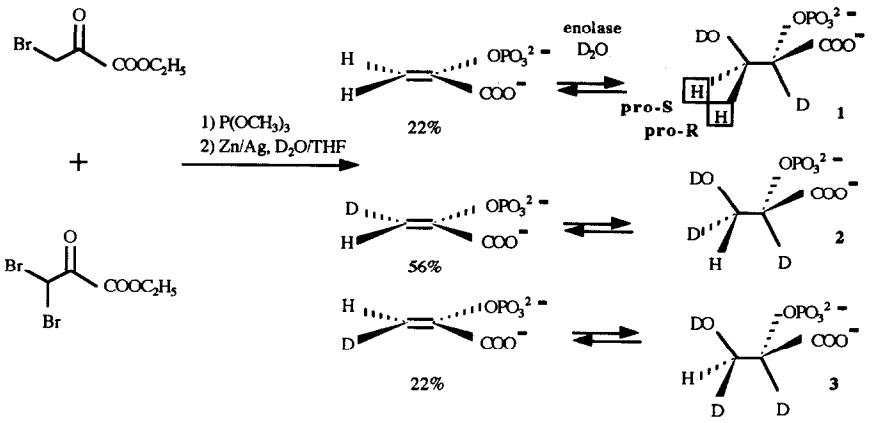
Results and discussion

In the case when one is able to label stereospecifically with deuterium the pro-R or the pro-S positions of the methylene group in 2-phosphoglycerate (2-PG), it becomes easy to assign the two protonic resonances. The methology used takes into account two opportunities :

- The first one uses the possibility to synthesize stereospecifically (E)- and (Z)-[3-^{2}H]-phosphoenolpyruvate (PEP) by means of a very short and efficient method described by Woodard et al [5].
- The enolase catalyses stereospecifically the transformation of PEP into 2-PG [6] (see scheme below). However, although the equilibrium constant value K = 5 is not in favour of 2-PG formation, it was supposed that sufficient amounts of the latter would be synthesized.



From the bromination of bromopyruvate a mixture of 2-PG and of the dibromo analogue was obtained. The separation of these two compounds was not performed because it was interesting for us to have the deuterated and the non-deuterated PEP in the same sample. The ^{1}H NMR spectra (see Figure 1b) show the following composition : PEP, 22%, (E)-[3-^{2}H]-PEP, 22%, (Z)-[3-^{2}H]-PEP, 56%. Taking into account the known stereospecificity of the enolase[6], the following mono and bideuterated analogues of 2-PG are expected (see scheme below) **1** : (2-R)-[2-^{2}H]-2-PG, **2** : (2-R, 3-S)-[2,3-^{2}H]-2-PG, **3** : (2-R, 3-R)-[2,3-^{2}H]-2-PG.



When equilibrium was reached the proton NMR spectra revealed the presence of new signals between 3.5 and 4 ppm (see Figure 1a and 1c) which can be easily assigned to the methylenic protons of 2-PG[1]. An AB system (^2J= -11.9 Hz) is due to the **1** derivative. It follows that the most intense signal at δ = 3.79 ppm and the less intense one at lower field (δ = 3.85 ppm) are responsible for the **2** and **3** species respectively.

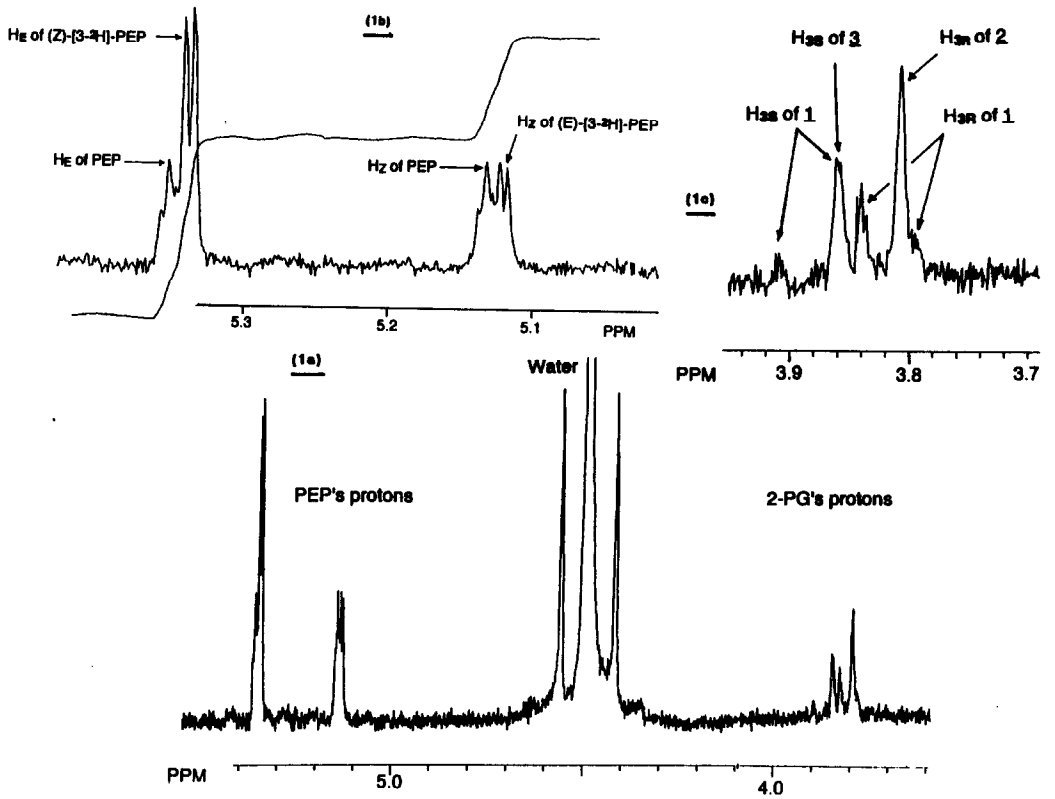


Figure 1- (1a): Proton NMR spectra of the mixture obtained after the action of the enolase on the deuterated and non deuterated PEP in D_2O, (1b): PEP's protons resonances only, (1c) : 2-PG's protons resonances only (chemical shifts in ppm; reference, 4,4-dimethyl, 4-silapentane sodium sulfonate salt : DSS)

This clearly means that in 2-PG the pro-S methylenic proton is shifted more than its pro-R neighbour. This attribution allowed us to follow the respective chemical shift variations of these protons in the presence of rare earth salts. As we have already reported[7], praseodium chloride is a very efficient complexing agent for the phosphate group and we have shown the possibility of individualizing the proton resonances for glucose-6-phosphate , glucose-1-phosphate and fructose-1,6-bisphosphate. 2-PG also exhibits a similar behaviour and the shift variations observed (see Figure 2) can be accurately discussed in conformational analysis terms.

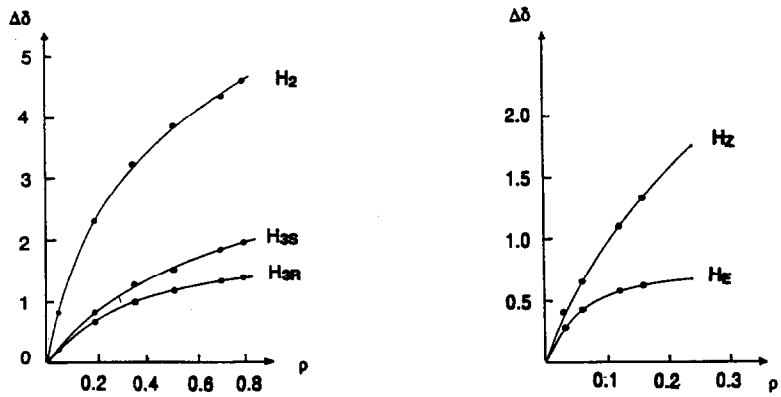
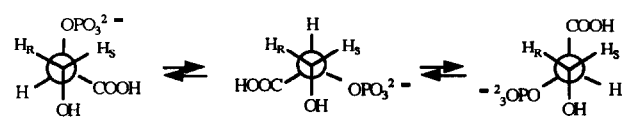


Figure 2: Proton Chemical Shift variations (in ppm) versus praseodium chloride concentrations ; ρ = [PrCl3] / [substrate] ;Δδ = δH(ρ=0) - δH(ρ=x) ; Left : 2-Phosphoglycerate and right : Phosphoenolpyruvate.

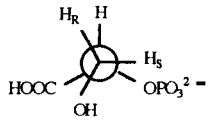
One could argue that the carboxylic group is also able to complex the praseodium salt. The study of the chemical shift variations of the ethylenic protons of PEP induced by the praseodium chloride complexing proves clearly that the phosphate function is much more active (see Figure 2) : the H_Z proton is shifted more than the H_E proton. A significant competition between the carboxylate and the phosphate functions would give, as a result, similar variations for the two protons. Figure 2 shows the influence of $PrCl_3$ on the proton shifts of 2-PG : a large effect is observed for H_2 and smaller ones for the two H_3. It is noteworthy to consider the stronger variations for the H_{3S} than the H_{3R} proton, a Δδ value ($\Delta\delta = \delta H_{3S} - \delta H_{3R}$) of 0.5 ppm was measured for a ρ value of 0.8 (see Figure 2). As the addition of increasing amounts of $PrCl_3$ also increases the initial pH of the solution, it is very likely that the effect of complexing produces a superior intrinsic Δδ value because, in our experiments, no correction of the pH was made. The reason which justifies this assertion is to be found in the work of Randall et al[4] who have studied the chemical shift variations of the protons of 2-PG with pH (see Table 1). A downfield shift was observed for the protons of 2-PG when lowering the pH but H_{3R} was shifted more than H_{3S} : variations of 0.17 and 0.05 ppm were respectively observed when going from pH = 10.8 to 1.0. Nevertheless, the influence of $PrCl_3$ on 2-PG proton chemical shifts clearly indicates a proximity of the H_{3S} proton towards the phosphate group. On this basis, structural considerations can be drawn but it must be pointed out that they will be limited to a pH range of 1 to 4 units. In effect, in a basic media, the complex with praseodium chloride precipitates and it is only soluble at acidic pH.

Three gauche conformers around the C_2-C_3 bond **1a, 1b, 1c** can be drawn for 2-PG:



A type **1a** conformation may be disregarded as very similar shift variations would be attempted. Futhermore, such a structure is inconsistent with the observed coupling constants (see Table 1). A type **1c** structure also seems impossible as the H_R proton in this situation should exhibit larger shifts than the H_S proton. Thus a type **1b** conformation has to be retained. The exact coupling constants are difficult to measure

at low pH because, in the abscence of praseodium chloride, the spectra were deceptively simple[1] and the presence of this salt drastically broadens the peaks. Nevertheless, no significant variation of the coupling constant values seems to occur between pH = 10.8 and pH = 5. Furthermore at low pH, the sum of the coupling constants $^3J_{H3S-H2}+^3J_{H3R-H2}$ was measurable and seemed to remain constant (7 to 7.5 Hz, see Table 1). This very probably means that the coupling constants mentioned above exhibit very low variations over a wide range of pH values (1 to 11). Taking in account this observation and the $^3J_{HH}$ values one can assume that the dihedral angle between H_{3S} and H_2 is very close to 90° (see below).



1 b

Such a conformation would be stabilized by hydrogen bonding between the carboxylic function and the alcohol function. An additional argument in favour of this conformation is also given by the larger shift variation with pH of H_{3R} compared to H_{3S}[1]. This indicates the proximity of H_{3R} to the carboxylic function which exerts very different effects with the modification of pH.

compound	pH	δ_{H3R}	δ_{H3S}	$^3J_{H3RH2}$	$^3J_{H3SH2}$	$^2J_{H3RH3S}$	Ref.[a]
2-PG	1	4.47	4.45	[7.0][b]		-12.1	1
	2.8	4.42	4.42	[7.4][b]		-11.7	1
	5.0	4.36	4.40	5.0	3.0	-	1
	10.8	4.30	4.40	5.4	2.9	-	1
(2-R,3-S)[2,3-2H]PG	7	3.79	-	-	-	-	this work
(2-R,3-R)[2,3-2H]PG	7		3.89	-	-	-	this work
(2-R)-[2-2H]PG	7	3.82	3.88	-	-	-11.9	this work

Table 1 : Proton NMR chemical shifts (in ppm) and coupling constants (Hz) for 2-Phosphoglycerate and of its deuterated analogues. [a]in Ref.1 the shifts were measured from external TMS and in this work from internal DSS ; [b]sum of the two coupling constants.

Experimental

- A $0.2.10^{-2}$ M solution in D_2O of a mixture of non deuterated PEP (22%), (Z)-[3-^{2}H]-PEP (56%) and of (E)-[3-^{2}H]-PEP was introduced into a NMR tube with 50 units of an enolase preparation from rabbit muscle (Sigma). The pH of the solution was adjusted to 7 with 1 N NaOD. The solution was allowed to stand for 24 H at room temperature . After this time equilibrium was reached .
- The NMR spectra were recorded on a Bruker WM 250 spectrometer operating at the nominal frequency of 250 MHz for the proton. The praseodium chloride was purchased from Ventron Karlsruhe-Germany.

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