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Supporting Information:

Materials

The following materials were purchased from the suppliers indicated: Hexokinase (EC 2.7.1.1 from bakers yeast), glucose 6-phosphate dehydrogenase (EC 1.1.1.49 from bakers yeast), 6-phosphogluconate dehydrogenase (EC 1.1.1.44 from yeast), glutamate dehydrogenase (EC 1.4.1.2 from bovine liver), phosphoriboisomerase (EC 5.3.1.6 from spinach), ATP (di-sodium salt), $NADP^+$ (di-sodium salt) from Sigma (Deisenhofen, Germany); alkaline phosphatase (EC 3.1.3.1 from calf intestine) from Boehringer (Mannheim, Germany); zinc chloride diethylether complex (2.2 M in dichloromethane) from Fluka (Neu-Ulm, Germany). 3,4-Dihydroxy-2-butanone 4-phosphate synthase was isolated from a recombinant *Escherichia coli* strain as described earlier (Richter et al., 1992). Enzyme activities are indicated in U where 1 U catalyzes the formation of 1 µmol product per minute.

Syntheses

[4-$^2H_1$]-3,4-dihydroxy-2-butanone 4-phosphate - The reaction mixture contained 20 mg [6-$^2H_1$] glucose (6R or 6S), 70 mM tris-(hydroxymethyl)-aminomethane (Tris) hydrochloride (pH 7.8), 100 mM dithiothreitol, 20 mM $MgCl_2$, 30 mM ATP, and 40 U hexokinase in a total volume of 6 ml. The mixture was incubated at 37 °C for 20 minutes. At this time at least 80 % of the glucose were converted to glucose 6-phosphate (monitored by the assay described below). Then, 60 µl of 10 M ammonium acetate, 600 µl of 1 M α-ketoglutaric acid, 600 µl of 20 mM $NADP^+$, and 75 µl of a solution containing 5 U of 6-phosphogluconate dehydrogenase, 12 U of glucose 6-phosphate dehydrogenase and 20 U of glutamate dehydrogenase were added. The total volume was 8.6 ml. The mixture was incubated at 37 °C for another 15 minutes and stored at 4 °C for 1.5 h. During the course of the reaction the pH was frequently readjusted to pH 7.8 by the addition of 1 M NaOH.

Subsequently, when no more glucose 6-phosphate could be detected and at least 65 % of the glucose were converted to ribulose 5-phosphate (monitored by the assays described below) 1 mg of recombinant 3,4-dihydroxy-2-butanone 4-phosphate synthase was added. The mixture was incubated at 25 °C for 2.5 h. Phosphoriboisomerase (10 U) was added, and incubation was continued for another 30 minutes.

$BaCl_2$ (1 M, 600µl) and trichloro acetic acid (2 ml, 15 %, w/w) were added. The mixture was shaken for 2 minutes and was neutralised with saturated $Ba(OH)_2$ (5 ml). The white precipitate was removed by centrifugation, and the supernatant was mixed with acetone to a final concentration of 80 %. The mixture was kept at -20 °C for 3 days. The white precipitate was harvested by centrifugation, washed twice with ethanol and dried over $CaCl_2$ in vacuo at 4 °C yielding about 60 % product as determined by enzymatic assay.

[4-$^2H_1$]3,4-dihydroxy-2-butanone - The dry barium salt of [4-$^2H_1$]3,4-dihydroxy-2-butanone 4-phosphate was suspended in 4 ml of 200 mM Tris hydrochloride, pH 7.8. In order to complete solubilisation, 20 µl of concentrated acetic acid were added. Barium ions were precipitated as barium sulphate by the addition of 500 µl Na_2SO_4 (0.4 M) and was removed by centrifugation. The supernatant was neutralized with 1 M NaOH (1.5 ml). Alkaline phosphatase (28 U) was added, and $MgCl_2$ and $ZnSO_4$ were added to final concentrations of 5 mM and 2 mM, respectively. The mixture (total volume 6.3 ml) was incubated at 37 °C for 1.5 h. The solution was saturated with NaCl and extracted in a liquid-liquid-extractor with 45 ml dichloromethane for 6 h. The organic layer was dried with $MgSO_4$ and concentrated in vacuo.

The crude product was purified by column chromatography on silica gel with hexane/ethyl acetate (1/4; v/v). Fractions containing the product (detected with TLC) were combined and concentrated in vacuo.

[5-$^2H_1$]2,2-Dimethyl-4-acetyl-1,3-dioxolane - [4-$^2H_1$]-3,4-dihydroxy-2-butanone was dissolved in 600 µl of dry acetone, and 40 µl of $ZnCl_2$/ether complex (2.2 M solution in di-

chloromethane) were added at room temperature. The reaction was followed by thin layer chromatography. When the starting material had disappeared, 300 µl of a K_2CO_3 solution (100 mg/ml) and 500 µl of water were added. The mixture was extracted four times with 2 ml of chloroform. The combined organic layers were dried with $MgSO_4$ and concentrated in vacuo.

Enzymatic assays

During the syntheses described above the progress of the reactions was monitored by the following assays.

Assay for glucose 6-phosphate - 5 µl of the reaction mixture were diluted with 45 µl of H_2O. 20 µl of the diluted reaction mixture were added to a solution containing 160 mM Tris hydrochloride, pH 7.8, 12 mM $MgCl_2$, 0.5 mM $NADP^+$ and 1 U of glucose 6-phosphate dehydrogenase in a total volume of 395 µl. The absorbance was monitored at 339 nm until it was constant. The concentration of glucose 6-phosphate was calculated using ε ($NADPH^+$) = 6,200 M^{-1} cm^{-1}.

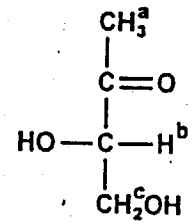
Assay for ribulose 5-phosphate - 15 µl of the reaction mixture were added to a solution containing 120 mM potassium phosphate, pH 7.0, 12 mM $MgCl_2$ and approximately 15 µg 3,4-dihydroxy-2-butanone 4-phosphate synthase in a total volume of 120 µl. The assay was incubated at 37 °C for at least 45 min. Then 10 µl of this mixture were added to a solution containing 16 mM EDTA, 200 mM potassium phosphate, pH 7.0, 1 mM 5-amino-6-ribitylamino-2,4(1H,3H)-pyrimidinedione (**3**) and approximately 100 µg of lumazine synthase in a total volume of 390 µl. The mixture was incubated at 37 °C for at least 30 min. Then the assay was diluted to 1/10 and the absorbance was monitored at 410 nm. The concentration of ribulose 5-phosphate was calculated using ε (lumazine) = 10,300 M^{-1} cm^{-1}.

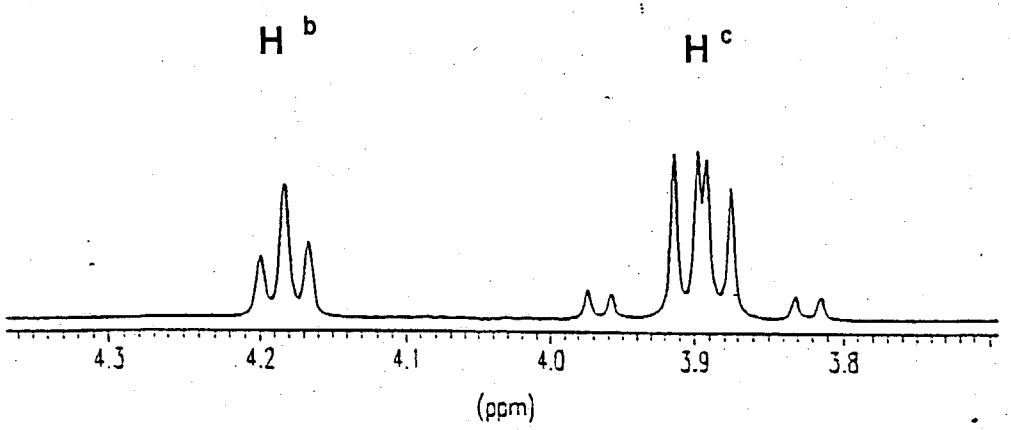
Assay for 3,4-dihydroxy-2-butanone 4-phosphate - 5 µl of the reaction mixture were added to a solution containing 100 mM potassium phosphate, pH 7.0, 2 mM EDTA, 1 mM 5-amino-6-ribityl-amino-2,4(1H,3H)-pyrimidinedione (**3**) and approximately 100 µg of lumazine synthase in a total volume of 395 µl. The mixture was incubated at 37 °C for at least 45 min. The assay was diluted to 1/10 and the absorbance monitored at 410 nm. The concentration of 3,4-dihydroxy-2-butanone 4-phosphate was calculated using ε (lumazine) = 10,300 M^{-1} cm^{-1}.

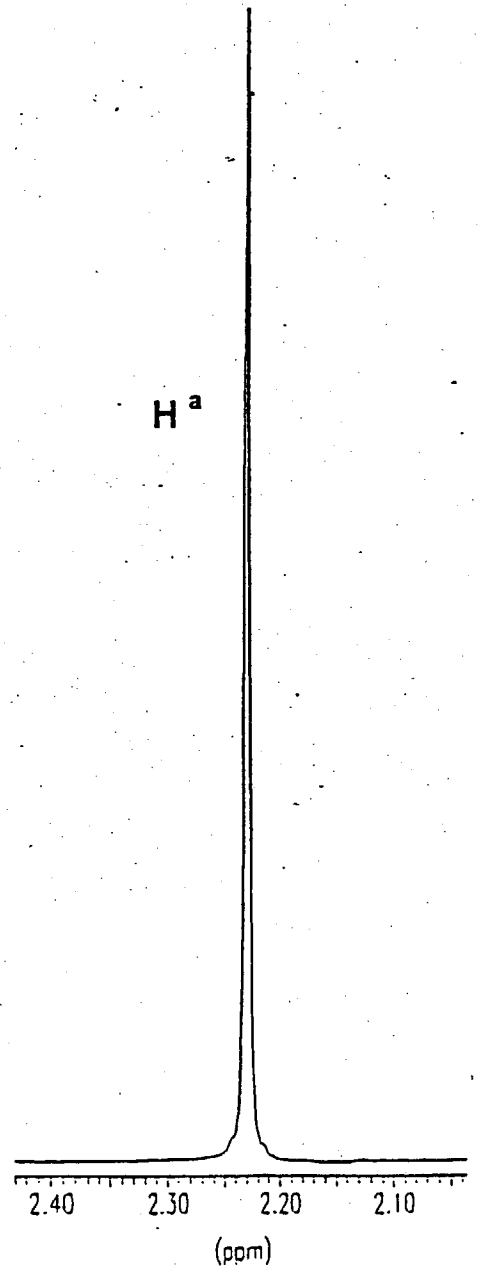
NMR spectroscopy

^{1}H NMR spectra were recorded at 200.13 and 500.13 MHz using AC200 and DRX500 NMR spectrometers from Bruker, Karlsruhe, Germany. ^{2}H NMR spectra were recorded at 76.77 MHz using a DRX500 spectrometer (Bruker, Karlsruhe, Germany) equipped with a $^{13}C/^{2}H/^{1}H$ triple resonance probehead and a ^{19}F lock. Typical parameters for ^{2}H NMR experiments were: repetition rate, 4.1 s; pulse width, 40°; sweep width, 7.6 kHz; raw data size, 32 K; temperature, 300 K.

^{1}H NMR Spectrum of Unlabelled 3,4-Dihydroxy-2-butanone

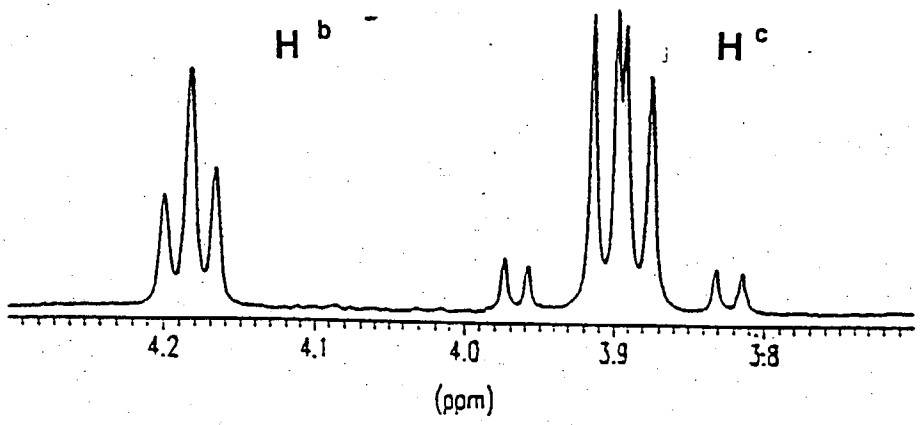




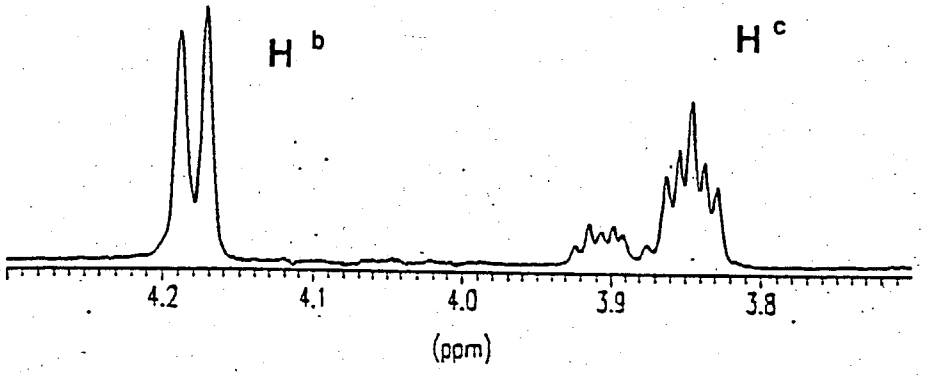


^{1}H NMR Spectra of Labelled and Unlabelled 3,4-Dihydroxy-2-butanone

3,4-Dihydroxy-2-butanone



[4-^2H_R]-3,4-Dihydroxy-2-butanone



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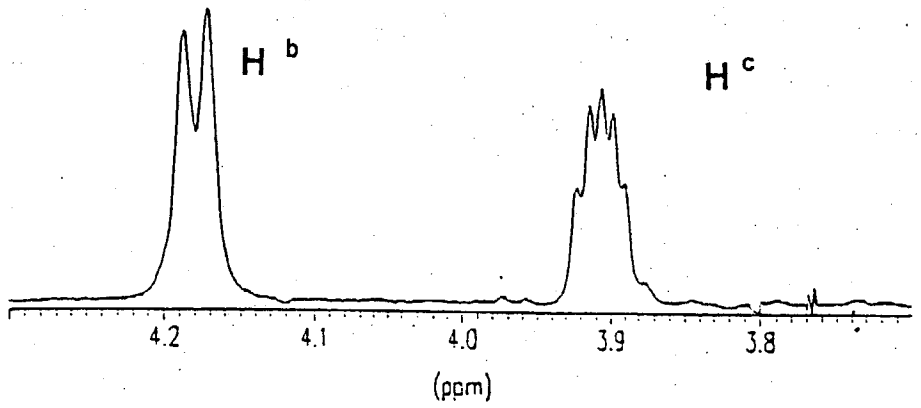
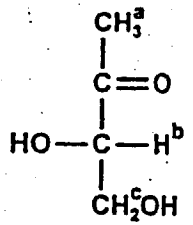
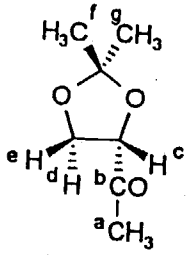




^{1}H NMR Spectrum of Unlabelled 2,2-Dimethyl-4-acetyl-1,3-dioxolane



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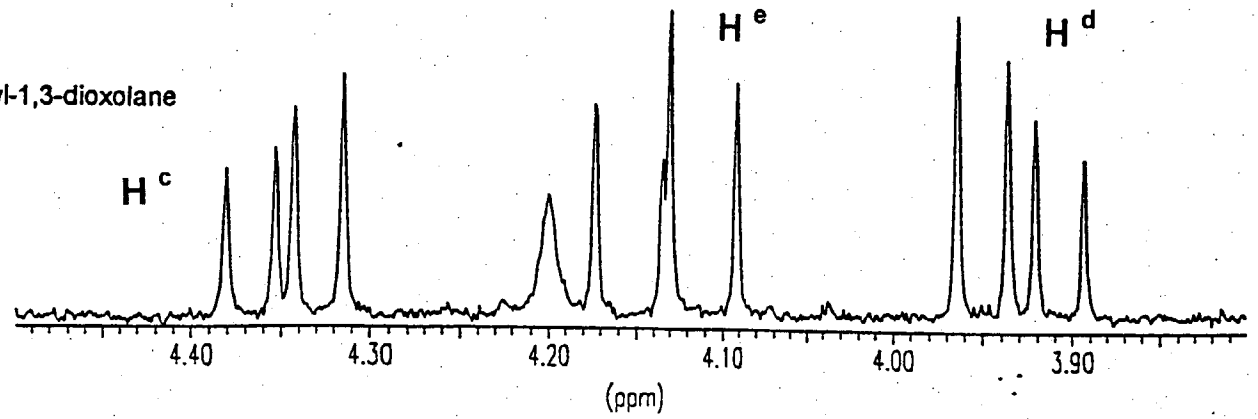
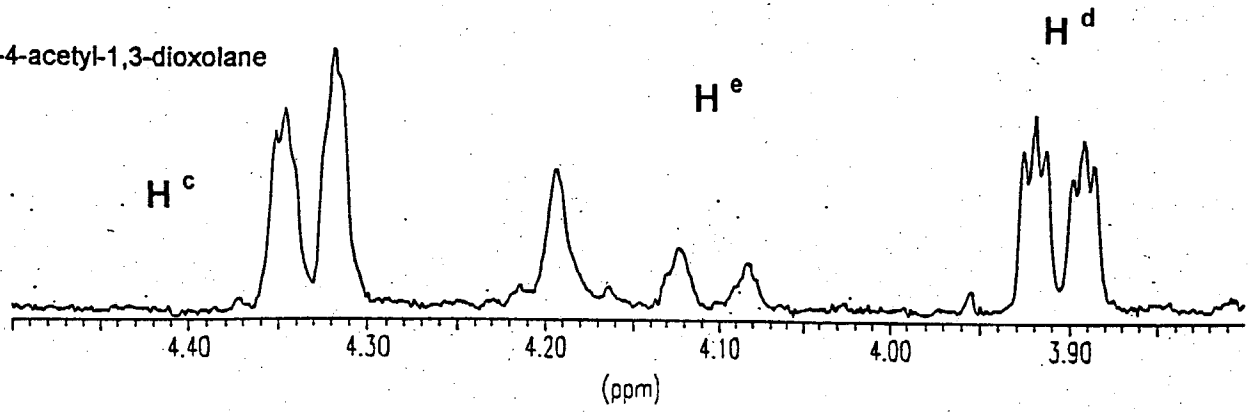
^{1}H NMR Spectra of Labelled and Unlabelled 2,2-Dimethyl-4-acetyl-1,3-dioxolane

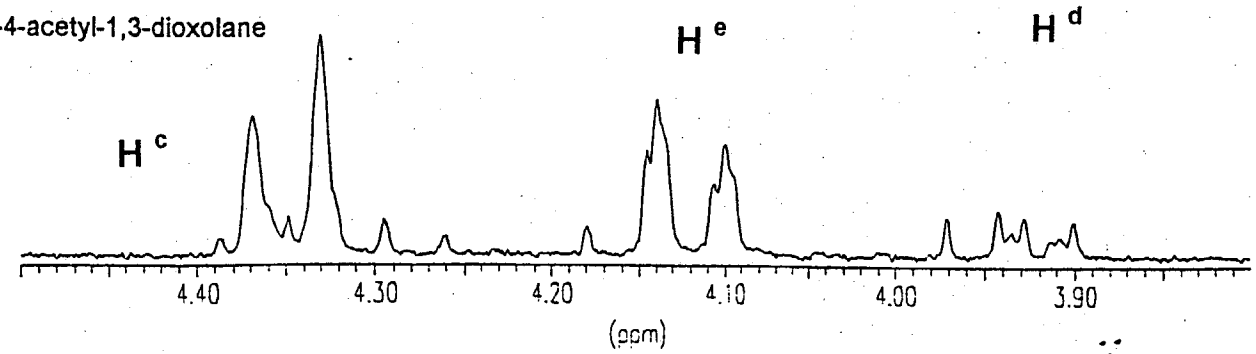
2,2-Dimethyl-4-acetyl-1,3-dioxolane

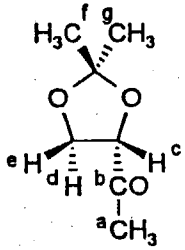


[5-^2H_R]-2,2-Dimethyl-4-acetyl-1,3-dioxolane



[5-^2H_S]-2,2-Dimethyl-4-acetyl-1,3-dioxolane





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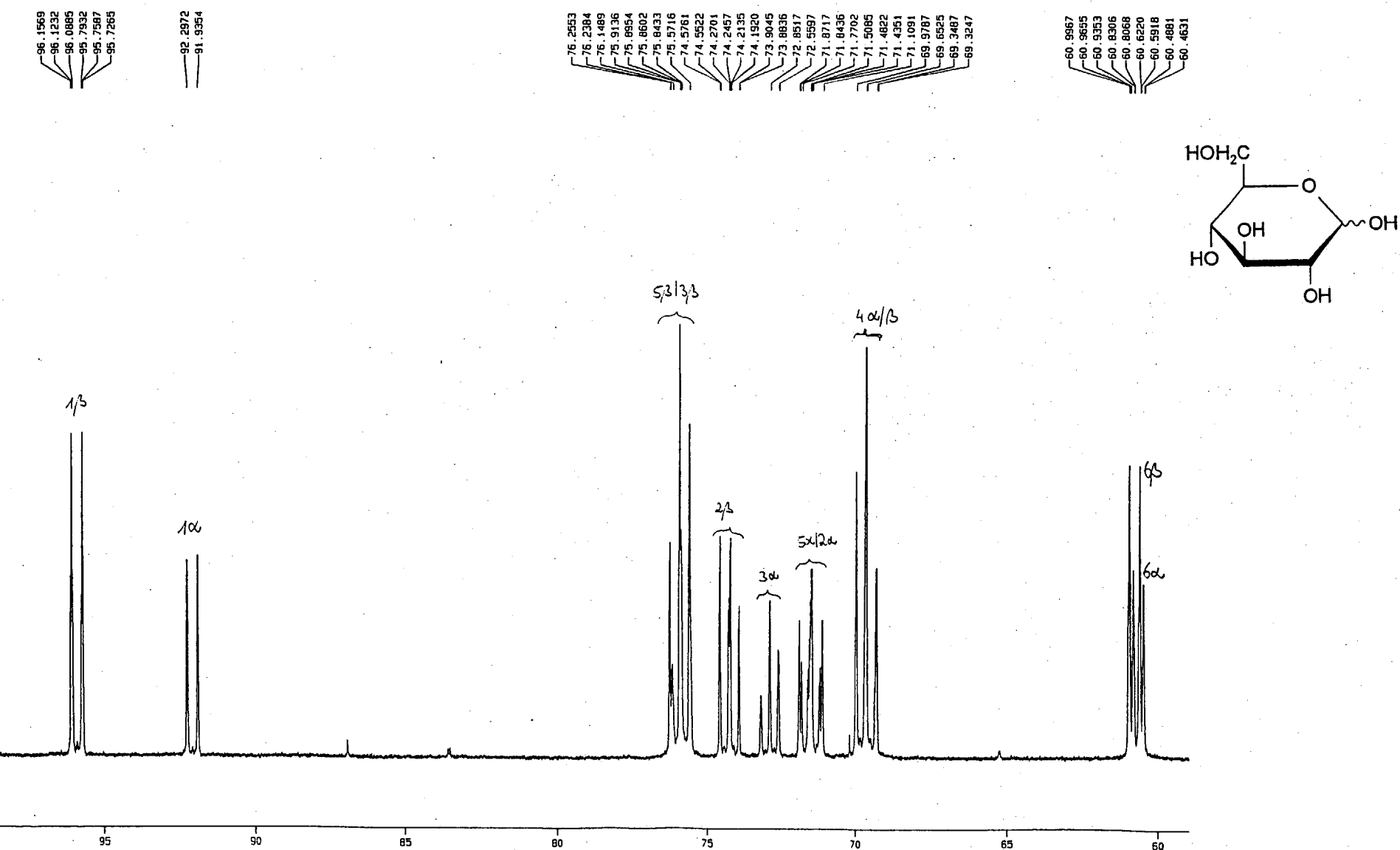
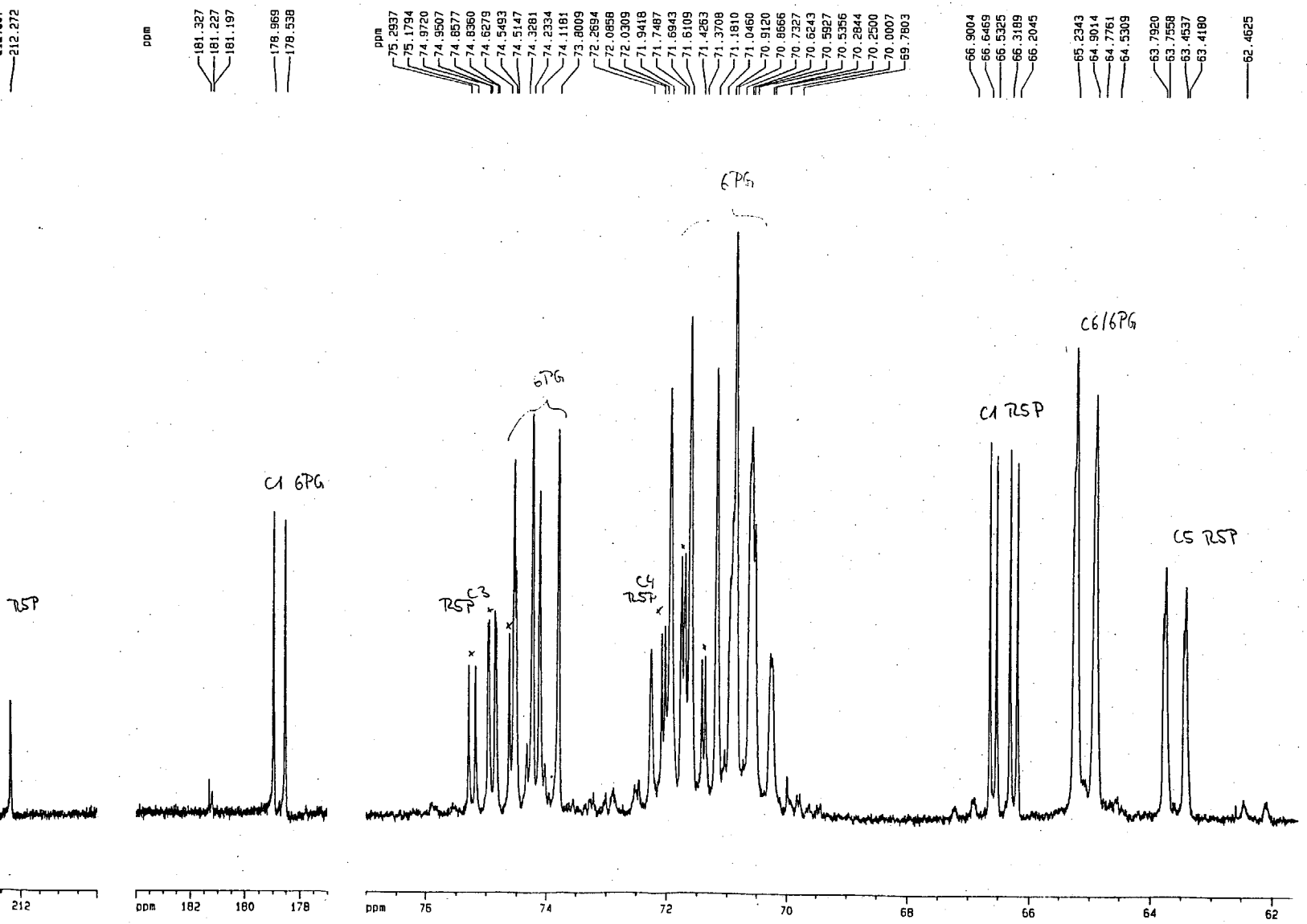
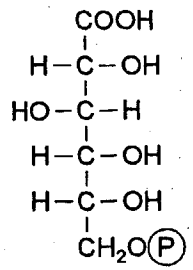
^{13}C NMR Spectrum of U-$^{13}C_6$-Glucose Before the Addition of Enzymes



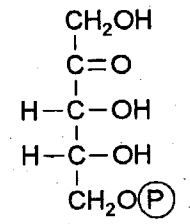
^{13}C NMR Spectrum of the Reaction Mixture Starting with U-$^{13}C_6$ -Glucose After 35 Minutes

(The reaction mixture contains the enzymes hexokinase, glucose 6-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase and glutamate dehydrogenase)
6PG = 6-phosphogluconate;
R5P = ribulose 5-phosphate





6PG



R5P

^{13}C NMR Spectrum of the Exclusive Formation of U-$^{13}C_5$ -Ribulose 5-Phosphate from U-$^{13}C_6$ -Glucose