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On the Mechanism of Baker's Yeast Mediated Synthesis of (R) *S*-Benzyl Thioglycerate. Experiments in Deuterated Water

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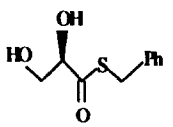
Summary Experiments in ca. 90% deuterated water in which (R) *S*-benzyl thioglycerate (**1**) is obtained in the presence of benzyl mercaptan in baker's yeast fermenting on D-mannose, D-glucose and D-fructose, respectively, gave rise to trideuterated materials. The extent of labelling at various positions and the stereochemistry depends upon the carbohydrate used as precursor, as shown by ^2H NMR studies onto the dioxolanes (**12**)-(**14**)

In the baker's yeast reductions of non-conventional substrates substantial amounts of D-glucose are added to the fermentation mixture as 'fuel' for the metabolic operations presiding over the regeneration of the required reduced nicotine cofactor(s). Carbon dioxide and ethanol are produced and, apart from some glycerol, no C-3 compounds amongst those identified as intermediates in the accepted glycolytic pathway (Scheme 2) is accumulated. It occured to us during experiments of b.y. transformation of non-conventional substrates, in which D-glucose and benzylmercaptan were present at the same time, to observe the formation of (R) *S*-benzylthioglycerate (**1**) possessing over 98% ee.



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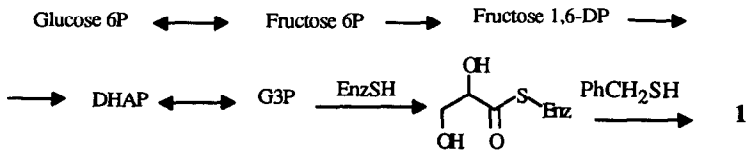
We tentatively invoked the intermediacy of the activated thioester of a 3C unit derived from glucose degradation transformed into the actual product isolated through a nucleophilic displacement by benzylmercaptan (Scheme 1).



Scheme 1

Feeding experiments with ^{13}C and ^{2}H regiospecifically labelled D-glucose support the derivation of (**1**). From the mechanism of glycolysis it is evident that all of the carbon atoms present in D-glucose are divided between the two 3C units glyceraldehyde 3-phosphate (G3P) (**5**) and dihydroxyacetone phosphate (DHAP) (**6**), both possible precursors of the glycerate (**1**). Starting with [1-^{13}C] and [6-^{13}C] D-glucose the thioglycerate obtained was regiospecifically deuterated at position 3 with a dilution of the label of 50% as expected from the preceding observations. The experimental results thus support the conversion of D-glucose-6-phosphate (**2**) into (**1**) through the pathway illustrated in Scheme 1, involving the intermediacy of fructose-6-phosphate (**3**), fructose-1,6-diphosphate (**4**) and of the two C-3 units, glyceraldehyde 3-phosphate (G3P) (**5**) and dihydroxyacetone phosphate (DHAP) (**6**), respectively. The sulphur nucleophile is expected to act onto the enzyme-bonded intermediate in the oxidation of G3P (**5**). The formation from the latter of (**1**) would simply require hydrolytic removal of phosphate. In the deuterium labelling experiments with 2-^{2}H-D-glucose, it can be expected that, according to the known mechanism of the glucose-6P to fructose-6P isomerization, only one hydrogen atom will be transfered stereospecifically from glucose C2 to fructose C1. This fact together with the degradative mechanism to the two 3C units allow recovery of only 25% of the label. The results obtained are in agreement also with these observations as far as the stereochemistry of the label at position 3 of (**1**) is concerned.

However, the extent of deuterium retention at position 3 of (**1**) in feeding experiments of D-glucose regiospecifically deuterated at positions 1, was lower than expected. This observation was tentatively explained supposing the presence in fermenting baker's yeast of a whole set of aldose-ketose isomerases, causing, in the 1,2-hydrogen shift, labilization and exchange with the solvent hydrogens of the label originally at positions 1 of the fed D-glucose.[3] Accordingly, in order to gain more information on the nature of the process leading to the incorporation in baker's yeast of benzyl mercaptan into (**1**) and to explain the results obtained with deuterated D-glucose we carried out experiments in ca. 90% deuterated water in which (**1**) was produced using, as precursors, D-glucose, D-fructose and D-mannose, respectively, and we now present the results obtained.

We were facilitated in assigning the stereochemistry of the label incorporated into (**1**) by the obtainment[2] from the latter of a 1,3-dioxolane, showing perfectly defined signals for the three relevant hydrogen atoms in the NMR spectrum (see experimental). Thus, the labelling pattern of educt (**1**) obtained in the three incorporation experiments is deduced straightforwardly from NMR studies on dioxolanes (**12**)-(**14**), whose

^{2}H NMR spectra are reported in Figure 1. The extent of deuteration at various positions of **(12)**-**(14)** is in the Table.

Table 1 Extent of deuteration at different positions of dioxolanes **(12)**-**(14)**, obtained in 90% deuterated water by b.y. fermentation of D-glucose, D-fructose and D-mannose in the presence of benzylmercaptan

entry	4.64 ppm H-4	4.03 ppm H_R-5	4.27 ppm H_S-5
(12)	**89.6%**	**27.8%**	**12.8%**
(13)	**89.9%**	**26.6%**	**14.4%**
(14)	**89.0%**	**24.2%**	**41.2%**

Position and extent of incorporation in **(1)** of deuterium in the experiments in which D-glucose, D-fructose and D-mannose were used as precursors, as deduced from the mode of labelling of products **(12)**-**(14)**, seem explicable through the intervention of three different enzymes. According to Scheme 2, the methylene group of **(1)** arises, at similar extent, from the two methylene groups at positions 1 and 6 of fructose 1,6-diphosphate **(4)**, *via* DHAP **(6)** and G3P **(5)**. It is well known[3] that of the two diastereotopic hydrogen atoms at position 1 of **(4)**, H_R arises by 1,2 shift from the hydrogen atom originally present at position 2 of D-glucose and that it underwent exchange with the medium hydrogens when fructose-6-phosphate **(3)** (the open forms are drawn in Scheme 2 for pictorial reasons) was formed from glucose-6-phosphate **(2)** under the catalysis of glucose-6-phosphate isomerase. Instead, H_S in **(3)** is the hydrogen atom originally present in position 1 of the D-glucose framework. However, this same hydrogen arises from position 2 of D-mannose when mannose-6-phosphate is catalytically converted into **(3)** by phosphomannose isomerase. In the two above mentioned 1,2-hydrogen shifts there is extended exchange with the solvent hydrogen atoms. Due to the different geometries of the intermediates, in the glucose-fructose and mannose-fructose isomerizations, the extent of the exchange reaches ca. 50% in the former instance and a much higher level in the second.[3] Thus, at the light of these considerations an explanation for the labelling pattern of **(12)**-**(14)**, obtained in the three experiments in deuterated water, is pictorially represented in a simplified manner in Scheme 3. When mannose-6-phosphate **(7)** is used as precursor, monodeuterated fructose-6-phosphate **(8)** is obtained, in which H_S becomes labelled with deuterium. Conversely, using glucose-6-phosphate **(2)** as precursor, H_R becomes labelled, as indicated in **(9)**. Indeed, the Figure shows that in **(14)**, formed from D-mannose, H_S is substituted for deuterium at the extent of 41.2%. Similarly, in **(12)**, formed in b.y. from D-glucose, H_R is substituted by ca. 27% for deuterium. However, the presence in **(14)** of ca. 24% deuterium at

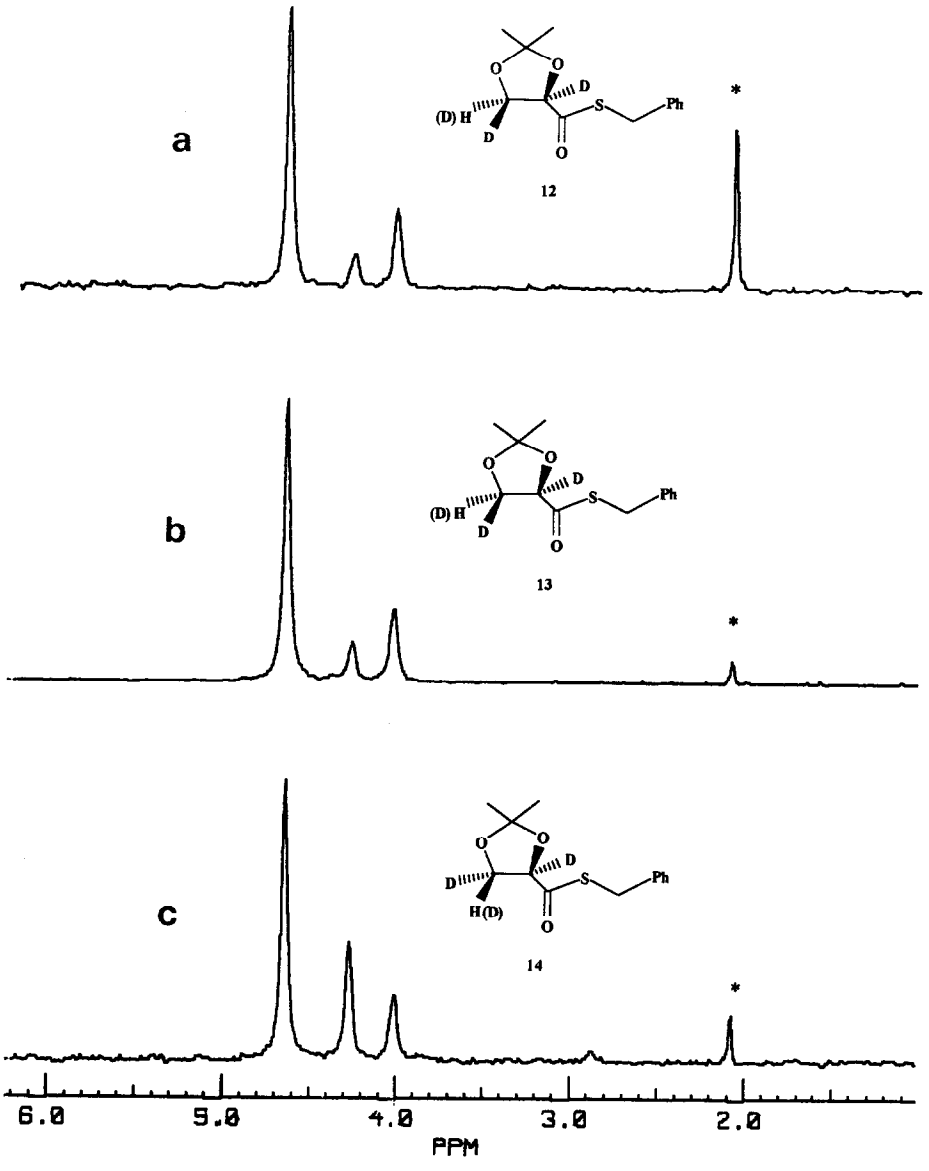
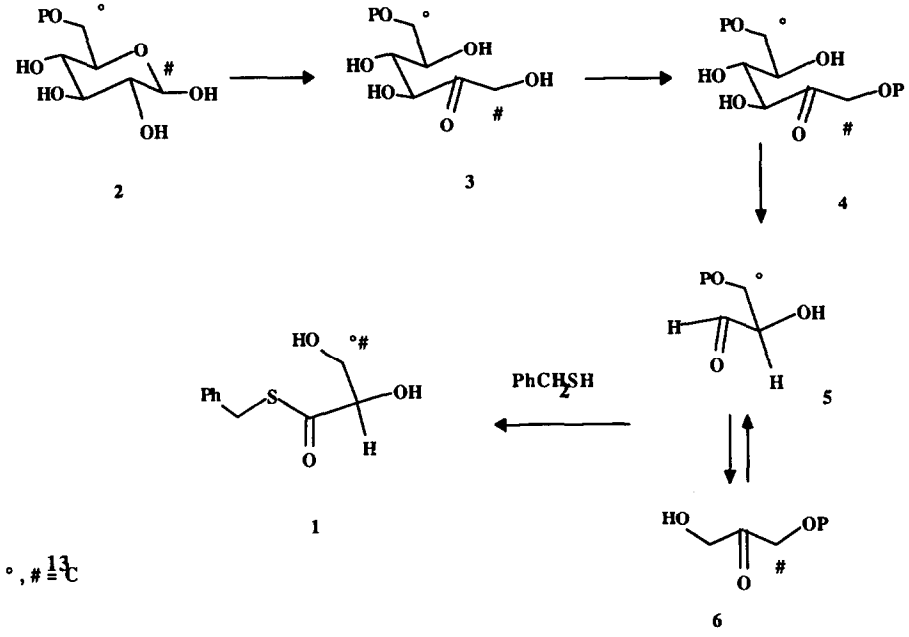


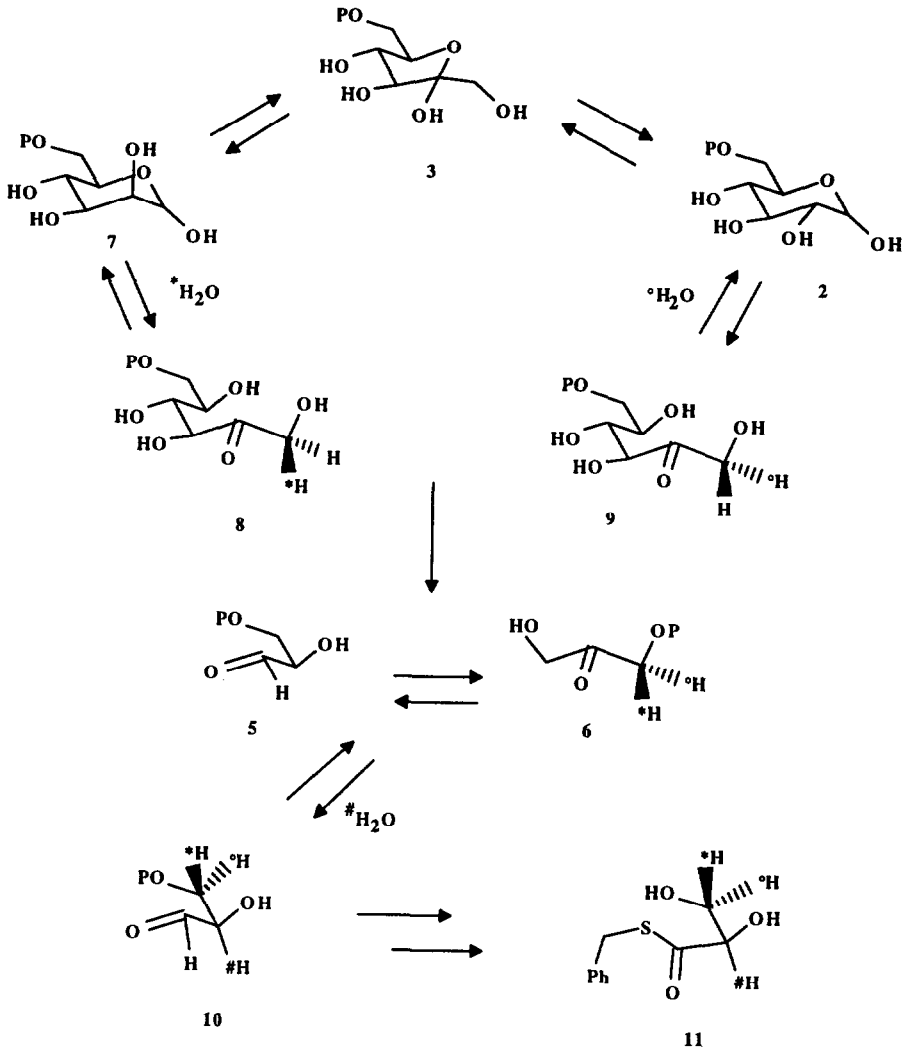
Figure 1. ^2H NMR spectra of 1,3-dioxolanes **(12)**-**(14)** obtained by b.y. fermentation in 90% deuterated water of (a) **D**-glucose **(12)**, (b) **D**-fructose **(13)** and (c) **D**-mannose **(14)**. The asterisks denote the natural abundance deuterium signals of the solvent (acetone).

position (3R) and in (**12**) of ca. 13% deuterium at position (3S) suggests that (**7**) and (**2**) are in equilibrium through fructose-6-phosphate, before this material is drained off to the 1,6-diphosphate. This phenomenon allows the formation, in the first experiment, close to (**8**) of some (**9**), whereas the opposite is true when glucose-6-phosphate is the precursor. These features are illustrated by the obtainment of (**13**) from D-fructose.



Scheme 2

The introduction in position 2 of the framework of (**1**) produced in b.y. in ca. 90% deuterated water of ca. 90% deuterium (cfr Figure 1 and Table 1), relative to the deuterium content of (**12**)-(**14**) is in agreement with the behaviour of triose-phosphate isomerase.[3] In this instance, the hydrogen atom stereospecifically removed from position 1 of the DHAP framework is exchanged with the solvent hydrogens before being delivered at position 2 to give G3P (**5**).



Scheme 3

In the oxido-reductive equilibrium between these species, doubly labelled **(6)** gives rise to triply labelled **(10)**, from which triply deuterated (R) *S*-benzyl thioglycerate **(11)** is actually obtained. It thus follows that the three deuterium atoms incorporated in derivatives **(12)-(14)** throughout the glycolytic pathway are inserted by exchange at three different stages, *i.e.* the (3R) deuterium in the glucose-fructose isomerization (°H in Scheme 3), the (3S) in the mannose-fructose isomerization (*H in Scheme 3), and, eventually at position 2 in the DHAP-G3P equilibration (#H in Scheme 3).

The above results obtained performing the transformation leading in b.y. to **(1)** in deuterated water thus appear complementary to those previously obtained[2] using regiospecifically ^{13}C and ^{2}H labelled D-glucose as precursor and show in a pictorial manner through the NMR spectra reported in Figure 1 the exchanges with the solvent hydrogens of the hydrogen atoms originally present at positions 1 and 2 of the hexose framework during the glycolytic degradation.

Finally, close to a mechanistic significance, this work could hold some synthetic interest. Indeed, since hydride reduction of asymmetrically labelled dioxolanes **(12)-(14)** leads to 1,2-isopropylidene glycerol bearing isotopic substitution at regio-and stereochemically relevant positions, the above experiments represent a further entry to asymmetrically deuterated glycerol.[4]

Experimental

General fermentation conditions. 5 g of benzyl mercaptan in 1 mL of EtOD were suspended in a solution of 200 mL of deuterated water (98%) and 40 g of sugar previously exchanged with 20 mL of D_2O for 12 h. To this mixture 45 g of dried yeast (VIVA S.T.L.) were added and the reaction was stirred under nitrogen at 25 °C for 18 h. Extraction with ethyl acetate and purification by silica gel chromatography gave from 150 to 200 mg of thioglycerate. Protection of the diol (DMP, benzene, TsOH) gave the required acetonide for NMR analysis.

The proton (300 MHz) and deuterium (46.1 MHz) spectra were obtained on a Bruker CXP 300 spectrometer. The ^{2}H NMR experiments were performed with the proton broad band decoupling on during the acquisition time and off during the relaxation time (3 s). Mass spectra were carried out on a Finnigan MAT TSQ 70 spectrometer under conditions of electron impact (70 eV).

NMR and MS data and assignments.

Dioxolane derivative of 1. ^{1}H NMR (acetone-d_6) δ 1.34 (3H, s, CH_3), 1.50 (3H, s, CH_3) 4.02 (1H, dd, H_R-5, $J_{5R,5S}$ = 8.8 Hz, $J_{4,5R}$=4.0 Hz), 4.10 (2H, s, SCH_3) 4.28 (1H,dd, H_s-5, $J_{4,5s}$=7.4 Hz), 4.64 (1H, dd, H-4), 7.20-7.35 (5H, m, C_6H_5). The *pro-R* and *pro-S* configuration of the H-5 methylene protons was established from the NOEs observed by selective irradiation of the two geminal methyl groups. Irradiation of CH_3 at 1.34 ppm induces enhancement of the signals of H-4 (3%, 4.64 ppm) and H_S-5 (2.8%, 4.28 ppm), while the irradiation of CH_3 at 1.50 ppm produces only a very small enhancement of H_R-5 (0.3%, 4.02 ppm).

EIMS analysis carried out on the unprotected diols related to **(12)** and **(14)** showed the molecular ion peaks

at m/e 213 (one deuterium atom incorporated) and 214 (two deuterium atoms incorporated).

Compound 12 (from feeding experiments with D-glucose). ^2H NMR (acetone) δ 4.65 (D-4), 4.28 (D_S-5), 4.03 (D_R-5) (see Figure (a) and Table). EIMS unprotected diol, m/z = 214 ($M^{+\cdot}$ doubly deuterated, 3); 213 ($M^{+\cdot}$ mono deuterated, 6); 124 (20); 92 (20); 91 (100).

Compound 13 (from feeding experiments with D-fructose). ^2H NMR (acetone) δ 4.64 (D-4), 4.27 (D_S-5), 4.02 (D_R-5) (see Figure (b) and Table).

Compound 14 (from feeding experiments with D-mannose). ^2H NMR (acetone) δ 4.63 (D-4), 4.28 (D_S-5), 4.01 (D_R-5) (see Figure (c) and Table). EIMS unprotected diol, m/z = 214 ($M^{+\cdot}$ doubly deuterated, 5); 213 ($M^{+\cdot}$ mono deuterated, 7); 124 (27); 92 (36); 91 (100).

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