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REDUCTION OF 1,1-DIDEUTERIO-2-METHYL-2-PENTEN-1-OL BY *BEAUVERIA SULFURESCENS*. MECHANISM OF THE MICROBIOLOGICAL REDUCTION OF α,β-UNSATURATED ALDEHYDES AND ALCOHOLS.

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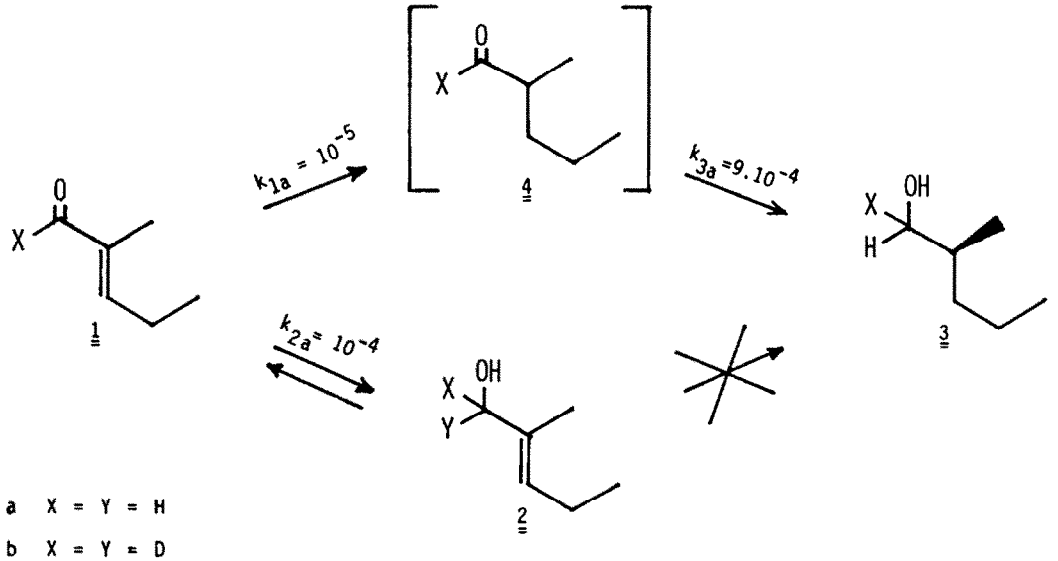
ABSTRACT : Reduction of 1,1-dideuterio-2-methyl-2-penten-1-ol by *Beauveria sulfurescens* leads to the corresponding saturated monodeuterated alcohol. This result allows the understanding of the mechanism of the microbiological reduction of α,β-unsaturated alcohols by *B. sulfurescens*. Thus, the stereochemical rule previously established for the reduction of α,β-unsaturated ketones can be carried out to the reduction of unsaturated aldehydes by *B. sulfurescens*.

In previous work (1) we studied the microbiological reduction of α,β-unsaturated aldehydes by *Beauveria sulfurescens* (ATCC 7159). The reaction gives the corresponding unsaturated and saturated alcohols. For 2-methyl-2-penten-1-al 1a, we proposed the reaction pathways shown in Scheme 1.



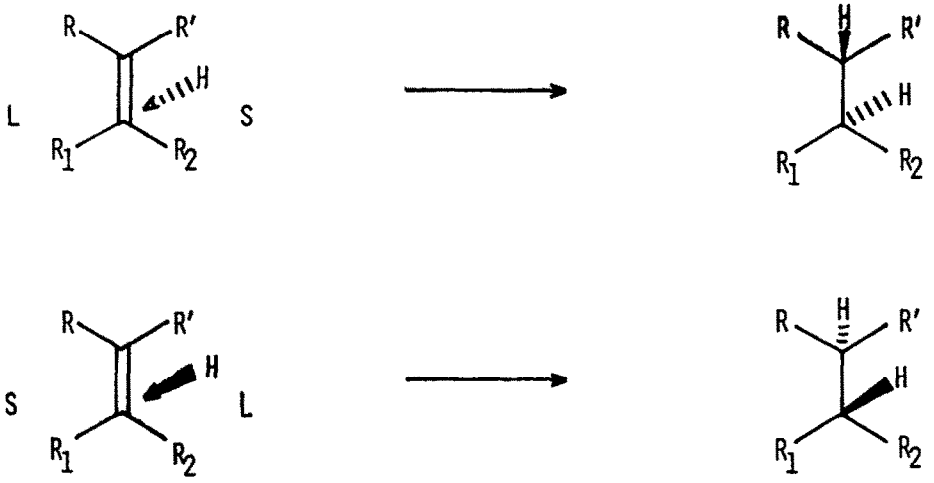
a X = Y = H

b X = Y = D

SCHEME 1

It was suggested that the final saturated alcohol could be obtained *via* two pathways. The first could involve the intermediate saturated aldehyde 4a which was not isolated. This pathway was envisaged to account for kinetic data which indicated a rate of reduction of 4a 10 times higher than its rate of formation. The second pathway would be through the intermediate unsaturated alcohol 2a. This seemed likely given that 2a is formed among the products of the reduction of 1a and that 2a can be reduced to 3a (1).

In the course of a study of the reduction of unsaturated ketones by *B. sulfurescens* (2) we proposed a stereochemical rule to account for the stereochemistry of the reduction products (Scheme 2).



SCHEME 2

One of the α-substituents R_1 or R_2 carries a carbonyl group, and one of the β-substituents R or R' is a hydrogen or a deuterium. The rule predicts that hydrogen will attack the double bond α to the carbonyl either from the front or the back depending on the relative bulk of R_1 and R_2. Now, if we apply this rule to the reduction of α,β-unsaturated aldehydes 1a, a problem arises. The saturated alcohol 3a has an asymmetric carbon with S configuration. If the above rule holds, then in the mechanism *via* 4a, $R_2(CH_3)$ must be bulkier than R_1(-CHO), which is reasonable. However, in the mechanism *via* 2a, $R_2(CH_3)$ should be bulkier than R_1 ($-CH_2OH$), which is unreasonable.

Whether or not 2a is actually reduced to 3a by the microorganism is not however proven. Indeed, it has been suggested (3), (4), that the reduction of unsaturated alcohols by microorganisms takes place *via* the corresponding unsaturated aldehyde. This pathway has in fact been evidenced by Gramatica *et al.*(4) for the microbiological reduction of a deuterated unsaturated alcohol. Then, the route that we proposed is 2 → 1 → 4 → 3.

In order to ascertain whether this pathway was involved in the reduction of unsaturated alcohols by *B. sulfurescens*, we studied the microbiological reduction of 1,1-dideuterio-2-methyl-2-penten-1-ol 2b. If 2b was directly converted into 3b, then the latter would contain two atoms of deuterium, whereas if 2b was first converted into 1b, then the final saturated product 3b would contain only one atom of deuterium.

The dideuterated substrate 2b was obtained by reduction of 2-methyl-2-pentenoic acid with $LiAlD_4$, and was left in contact with *B. sulfurescens* for 4 days. We obtained a mixture of saturated alcohol (51 %) and unreacted starting material (49 %), which were separated by preparative gas chromatography. Mass spectrometry revealed that the 2-methylpentanol 3b obtained was 98 % monodeuterated (the remaining 2 % was probably dideuterated), thus ruling out the direct route 2b → 3b.

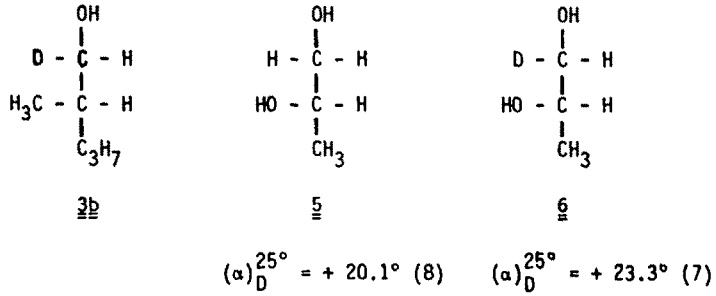
Alcohol 3b has two diastereoisomeric forms. The mixture was prepared by reduction of (±) 4a with $LiAlD_4$. Its 400 MHz ^1H NMR spectrum showed two quadruplets for the C1 protons geminal to OH of each diastereoisomer : δ = 3.35 ppm, J = 7 Hz for one and δ = 3.45 ppm, J = 5.9 Hz for the other (intensity 1/2 H for each diastereoisomer). The same values were found for the diastereotopic pro-

tons of the nondeuterated compound 3a with a geminal coupling of 10 Hz. The spectrum of alcohol 3b obtained by microbiological reduction was that of a single diastereoisomer (δ = 3.35 ppm, J = 7 Hz).

The enantiomeric purity of the saturated alcohol 3a also obtained by microbiological reduction was determined by ^{1}H NMR in the presence of a chiral europium derivative (2). The signal due to the 2-methyl was a single doublet, whereas two doublets are observed with the racemic alcohol. The optical purity was greater than 95 %. An enantiomeric purity of the same order for the 2-carbon of 3b (S configuration) was admitted. Thus alcohol 3b may be assumed to be a single enantiomer.

The monodeuterated saturated alcohol 3b had an optical activity $(\alpha)_J^{25°}$ = -7.8°, while the undeuterated derivative had $(\alpha)_J^{25°}$ = -13°. This difference is attributable to the additional asymmetric carbon present in the monodeuterated derivative (carbone 1).

This 1-carbon can be taken to have the S configuration as in general for the reduction of 1-deuterated aldehydes under these conditions (6). Alcohol 3b is shown in Fisher projection in Scheme 3.



SCHEME 3

The primary deuterated alcohols have a weak optical activity of the order of -0.5° for the S isomers (6). However, few examples of 1-deuterated alcohols bearing an additional asymetrical carbon have been described. One such is propylene glycol 5 (8) and its deuterated derivative 6 (7). The difference in optical activity between 5 and 6 is + 3.2° ; that between 3a and 3b is + 5.2°, i.e. of the same sign and order of magnitude.

The unreacted 2-methyl-2-penten-1-ol recovered from the reaction mixture had retained both its deuterium atoms, as established by ^{1}H NMR and mass spectrometry. This is surprising since if an equilibrium is set up between the unsaturated alcohol and the corresponding unsaturated aldehyde, some monodeuterated starting material should be recovered. Grammatica *et al.* (4) in the case of cinnamic alcohol recovered 86 % of dideuterated alcohol from a substrate containing 95 % dideuterated alcohol, for an equilibrium between the two ethylenic species considered as fast step. Apparently then, the equilibrium between 2b and 1b is so slow that the quantity of monodeuterated 2b recovered is below the detection thereshold (less than 5 % perhaps).

In the course of the study of the microbiological reduction of α,β-unsaturated aldehydes (1), we determined the rate constants for the reaction on undeuterated substrates involved in the two pathways initially postulated (1a → 4a → 3a and 1a to 2a to 3a). The values obtained are given in Scheme 1. The rate constant for the step 2a → 3a (k_{4a} = 2 x 10^{-5}sec^{-1}) was in fact an overall rate constant and should not therefore exceed the rate for the slowest step in the pathway 2a → 1a → 4a → 3a. The slowest of these three steps is the step 1a → 4a (k_{1a} = 10^{-5}sec^{-1}). Differences observed here with deuterated material may be attributed to an isotope effect due to deuterium. Reactivities of 2b and 1b should be different, leading to k_1/k_2 ratios different from those obtained with 2a and 1a. In addition, the kinetic determinations are somewhat inaccurate as they correspond to different experiments ; the amount of mycelium is not readily reproducible.

To conclude, the study of the microbiological reduction of 1,1-dideuterio-2-methyl-2-penten-1-ol by *B. sulfurescens* reported here provides an insight into the mechanism of the microbiological reduction of aldehydes and unsaturated alcohols. In particular, the stereochemical rule established to account for the stereochemistry of the reduction of α,β-unsaturated ketones is evidently valid

for that of α,β-unsaturated aldehydes and can moreover be extended to cover reactions previously reported elsewhere. Thus, geranial and geraniol (probably *via* geranial) are reduced by *Saccharomyces cerevisiae* to give (R) citronellol. Neral and nerol (Z isomer) should, according to the rule, give (S) citronellol. In fact, they give a mixture of (R) and (S) isomers in the ratio 6/4. This may be due to partial *cis-trans* isomerization of neral which appears to be an intermediate in the microbial conversion of nerol to citronellol (5).

EXPERIMENTAL

NMR spectra were recorded on a Jeol CX60 instrument with TMS as internal standard, in $CDCl_3$. Chemical shifts are in ppm. Preparative gas phase chromatography was carried out using a Varian 3 P90 instrument with catharometer, fitted with a 6 m aluminium column 3/8 in. in diameter filled with 20 % Carbowax 20M on Chromosorb W. The carrier gas was hydrogen (flow-rate 4 ml/sec). Oven temperature was 110°C.

Optical activities were determined using a Perkin-Elmer 141 polarimeter at 25°C at the wavelenghth of the mercury J line (λ = 578 nm).

Mass spectra were determinated by the Central Analytical Laboratory of the Centre National de la Recherche Scientifique at Solaize.

1,1-dideuterio-2-methyl-2-penten-1-ol 2b

A suspension of 1 g (23.8 mM) of $LiAlD_4$ in 40 ml of ether was placed in a flask fitted with a condenser and a tap-funnel. 2.25 g (19.7 mM) of 2-methyl-2-pentenoic acid in 35 ml of ether was added dropwise at a rate such that the ether refluxed gently. Once all the acid had been added, the mixture was refluxed for 2 hours with stirring. After cooling, water was added dropwise followed by 10 % sulfuric acid. The organic and aqueous phases were separated and the latter extracted several times with ether. The ether extracts were combined, washed with sodium bicarbonate solution and dried over sodium sulfate.

The extracts were evaporated to dryness giving 1.5 g of 1,1-dideuterio-2-methyl-2-penten-1-ol (yield 60 %).

^1H NMR : 0.90 (triplet 3H) ; 1.60 (singlet 3H) ; 1.70 to 2.30 (multiplet 2H) ; 3.30 (singlet 1H) ; 5.32 (triplet 1H).

Mass spectrum : mass peak 102 (very weak peaks at 101 and 100).

Microbiological reduction of 1,1-dideuterio-2-methyl-2-penten-1-ol.

Beauveria sulfurescens (ATCC 7159) was grown as previously described (2).

A solution of 1 g of 1,1-dideuterio-2-methyl-2-penten-1-ol in 3 ml of dimethylsulfoxide was added to 1.7 l of a 24 h old culture of *B. sulfurescens* in a 2 l fermentor under an aeration flow rate of 10 ml/l/min. After 4 days, the contents of the fermentor was filtered, and the filtrate saturated with ammonium sulfate and extracted 4 times with ether. The ether extracts were collected, dried over sodium sulfate and evaporated to dryness at 42°C.

The residue obtained consisted of 51 % of 2-methylpentan-1-ol and 49 % of unreacted 2-methyl-2-penten-1-ol. Yield about 90 % was determined by addition of an internal standard. These were separated by preparative gas phase chromatography under the conditions described above:

1-deuterio-2-methylpentan-1-ol 3c

Retention time 4 min. $(\alpha)_D^{25°} = -7.8°$. (C = 0,018 $CHCl_3$).

^1H NMR : 0.80 to 1.80 (multiplet 11H) ; 2.15 (singlet 1H) ; 3.37 (doublet 1H).

Mass spectrum : mass peak 103 (very weak peaks at 104 and 102).

1,1-dideuterio-2-methyl-2-penten-1-ol

Retention time 6 min. ^1H NMR and mass spectra identical to those of the starting material.

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