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MICROBIAL REDUCTION OF α,β-UNSATURATED CARBONYL COMPOUNDS: A GENERAL PROPERTY ?

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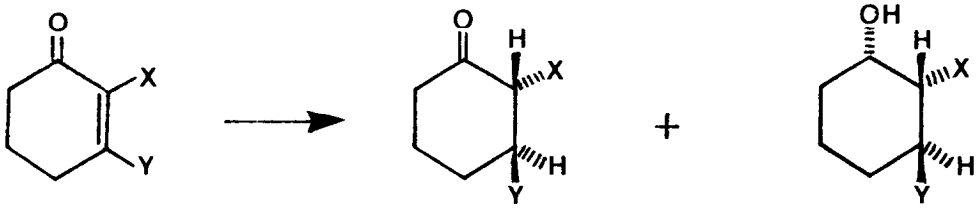
Abstract : *The double bonds of cyclohexenone and methylcyclohexenone were reduced by a strain of Clostridium and by various strains of Streptomyces. These results and those observed previously show that this reduction is widespread among microorganisms.*

The microbial reduction of the carbon-carbon double bond of α,β-unsaturated carbonyl compounds has been described for numerous substrates (1). Many of these reduction reactions are highly specific, e.g. the reduction of the 4,5 double bond in steroids (2,3). We have already reported results for the microbial reduction of carbon-carbon double bonds in small α,β-unsaturated ketones (10 or so carbons), in particular variously substituted cyclohexenones.



The reducing microorganisms studied were *Beauveria sulfurescens* ATCC 7159 (4,5) under microaerophilic conditions and various other *fungi* (6) under normal conditions of aeration. The reduction reaction bears the following characteristics :

- The substituent X must not be too large. When X is an ethyl group, the reaction is appreciably slowed.
- The substituent Y must be hydrogen.
- The configuration of the asymetrical carbon formed α to the carbonyl may be predicted by a simple rule (4).
- Addition of hydrogen is *trans*.

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We report here results for the reduction of cyclohexenones by *Clostridium* and *Streptomyces*. The characteristics of the reduction by these microorganisms are identical to those given above, suggesting that this reaction is a widespread property among eukaryotic and prokaryotic organisms.

2. Materials and Methods

The preparation of the substituted cyclohexenones used has already been described (4). Cyclohexenone was commercial.

2.1. The nutrient medium for *Clostridium* sp La 1, DSM 1460 was ; crotonic acid 6 g, NaOH (pellets) 0.3 g, Difco casamino acids 1 g, yeast extract 1 g, sodium thioglycolate 0.5 g, $(NH_4)_2HPO_4$ 150 mg, K_2HPO_4 100 mg, $MgCl_2\ 6H_2O$ 33 mg, $CaCl_2.2H_2O$ 40 mg, NH_4Cl 50 mg, $(NH_4)_6Mo_7O_{24}.4H_2O$ 10 mg, $MgSO_4.7H_2O$ 0.6 mg, $MnSO_4.2H_2O$ 0.4 mg, resazurin 1 mg, p-aminobenzoic acid 0.8 mg, in 1 l of distilled water.

100 ml portions of this medium were placed in 100 ml conical flasks stoppered with rubber septa. After sterilization the pH of the medium was adjusted to 6.8-6.9 by addition of 0.8 ml of 50 % K_2CO_3 solution per 100 ml of medium. 0.5 ml of 2 x 10^{-5} M $FeSO_4$ solution was also added. The flasks were shaken at 35° C, and the optical density at 578 nm monitored. Once this value had reached 1.5, the flasks were used to seed 500 ml screw-top flasks containing 450 ml of the same medium. These flasks then underwent the same procedure as the previous ones.

0.1 ml of cyclohexenone was added to the medium about 20 hours after seeding, the substrate was then left in the medium for a further 24 hours. With 2-methyl cyclohexen-2-one, sterile hydrogen was bubbled through the culture medium 15 hours after adding the substrate, the substrate was then left in the medium for a further 33 hours.

Extraction and purification of reaction products was performed as previously described (4).

2.2. *Streptomyces chartreusis* NRRL 3882 was grown in the following medium ; glucose 10 g, yeast extract 1 g, meat extract 1 g, casamino acids 4 g, in 1 l of tap water. pH was adjusted to 7.2 500 ml conical flasks were used, containing 100 ml of medium placed in a rotary shaker at 27° C. 50 mg of substrate was added to each flask after 24 hours growth. The culture was left under agitation for 48 hours.

2.3. *Streptomyces griseus* ATCC 10137 was grown in ; glucose 20 g, soybean meal 5 g, yeast extract 5 g, NaCl 5 g, K_2HPO_4 5 g in 1 l of tap water. The pH was adjusted to 7.2. Culture and reaction conditions as in 2.2.

2.4. *Nocardia rubra* ATCC 15906 was grown in ; yeast extract 1 g, meat extract 4 g, Difco casamino acids 4 g, glucose 10 g in 1 l of tap water. The pH was adjusted to 7.2. Culture and reaction conditions as in 2.2.

3. Results and discussion

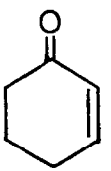
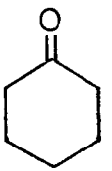
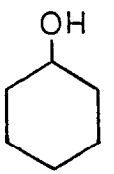
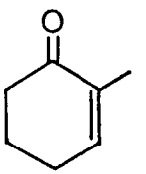
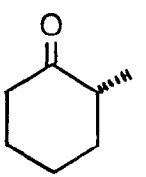
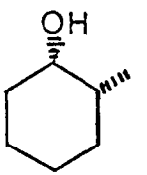
The strain of *Clostridium* sp La 1 (DSM 1460) used by Simon et al. (7) was chosen since its biochemical properties had been studied, enzymes isolated and the timing of their appearance during growth ascertained. Reduction of unsaturated acids had been studied with washed cells under strictly anaerobic conditions. Our reductions were performed with growing cells under anaerobic conditions. Cyclohexenone 1a (X = Y = H) was totally reduced in 24 hours, but here no cyclohexanol was obtained, as was the case with the *fungi* previously studied. Presumably, the enoate reductase evidenced by Simon is involved and no alcohol deshydrogenase. 2-deutero cyclohexenone 1b (X = D, Y = H) and 3-deutero cyclohexenone 1c (X = H, Y = D) gave the corresponding saturated ketones 2b and 2c, with the same stereochemistry as that observed with the *fungi*. 2-Methyl cyclohexenone 1d (X = CH_3, Y = H) gave the corresponding saturated ketone 2d, without any saturated alcohol, with yields lower than those obtained with the *fungi*. 10 % conversion was achieved under the conditions described above, and 30 % in the presence of hydrogen.

Several strains of actinomycetes were also studied. Results for three of these are given in Table I, for the reduction of cyclohexenone 1a and 2-methyl cyclohexenone 1d.

S. chartreusis produces the ionophorous antibiotic A23187 (calcimycin) (8), the biosynthes is of which involves reduction steps. The strain of *S. griseus* had been used by Rosazza (9) for the hydroxylation of various substrates. *N. rubra* was shown to degrade natural rubber (10). These strains thus possess widely different biochemical properties. The three actinomycetes strains cited in Table I completely reduced cyclohexenone 1a and yielded varying amounts of cyclohexanol 3a. In addition, they also reduced 2-methyl cyclohexenone 1d with yields lower than those obtained with the fungi. 2-Methyl cyclohexanol 3d was also obtained. The stereochemistry of the two reduction products 2d and 3d was the same as that obtained with *B. sulfurescens*.

Several reactions of the same type have been described with molecules of the same size. Yeasts reduce 3-methyl cyclohexenone (11) and isophorone (12). Finally, another yeast, *Rhodotorula mucilaginosa* reduces car-3-en-2,5 dione (13). However, the enzymes from yeasts appear to be less sensitive to steric hindrance than those from the microorganisms described above. *Geotrichum candidum* reduces a ketal of an unsaturated aldehyde (14) and carvone is reduced by *Pseudomonas ovalis* (15), *Streptomyces* and *Nocardia* (16). In this last case, a mixture of stereoisomers is obtained, whereas the reaction with *B. sulfurescens* (4) and other *fungi* (6) is stereospecific.

Table I : Microbiological reduction of cyclohex-2 en-1 one and 2-methyl cyclohex-2 en-1 one by some micro-organisms in 48 hours

	 1a	 2a	 3a	 1d	 2d	 3d
Beauveria sulfurescens ATCC 7159 {4}	0	40	45	0	30	55 (a)
Penicillium decumbens NRRL 742 {6}				0	10	85 (a)
Clostridium sp. La 1 DSM 1460	0	100	0 (c)	70	30	0 (b)
Streptomyces chartreusis NRRL 3882	0	60	40	65	25	10 (b)
Streptomyces griseus ATCC 10137	0	30	70	60	30	10 (b)
Nocardia rubra ATCC 15906	0	100	0	60	25	15 (b)

(a) yields of recovered products.

(b) yields calculated by GPC.

(c) reaction time 24 hours.

Thus, the two reduction reactions involving carbon-carbon double bonds described appear to be widespread among eukaryotic and prokaryotic microorganisms. The enzyme characteristics seem to be similar ; 2-methyl cyclohexanone is somewhat less completely reduced by prokaryotic species. Nevertheless, the stereochemical characteristics of the reduction are identical.

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