Enzymatic Production of Saturated Ketones from Allylic Alcohols

By SHELDON W. MAY,* MICHAEL S. STELTENKAMP, KENWOOD R. BORAH, ANDREAS G. KATOPODIS, and JOAN R. THOWSEN

(*School of Chemistry, Georgia Institute of Technology, Atlanta, Georgia* 30332)

Summary Allylic alcohols (*e.g.*, oct-1-en-3-ol) are converted, in preparative yields, into saturated ketones by *Pseudomonas oleovorans* and this process is shown to involve independent alcohol dehydrogenase and olefin reductase enzymes.

RECENT work with the *Pseudomonas oleovorans* olefin epoxidation system[1] has provided specificity and stereochemical findings and configurational information from studies with deuteriated substrates have been cited in evaluating mechanistic proposals.[2] The known reactivity and directive effects of allylic alcohol substituents on chemical epoxidizing systems[3,4] led to our examination of the reactivity of allylic alcohols in crude extracts of *P. oleovorans*. Unexpectedly, we observed the production of saturated ketones from these substrates in high yields, and we report here the various substrates studied and the resolution of the enzymatic steps in this process.

TABLE. Identification and quantification of products from enzymatic epoxidation, dehydrogenation, and reduction reactions[a]

Substrate	Product	Yield (mg ml^{-1}) *vs.* reaction time 10 h	20 h	40 h
Octa-1,7-diene	7,8-Epoxyoct-1-ene[b]	0·80	1·50	1·40
Oct-1-en-3-ol	Octan-3-one[b]	0·50	1·00	1·10
	Octan-3-ol[b]	<0·10	0·20	0·20
	Oct-1-en-3-one[b]	—	0·50	0·10
Oct-1-en-3-one	Octan-3-one[c]	2·00	2·50	3·00
Pent-1-en-3-ol	Pentan-3-one[c,e]	0·20	0·40	0·40
But-1-en-3-ol	Butan-2-one[c,e]	—	0·20	0·25
2-Methylhept-1-en-3-ol	2-Methylheptan-3-one[b]	0·10	0·30	0·40
	2-Methylhept-1-en-3-one[c]	<0·10	0·15	0·20
2-Methylhept-1-en-3-one	2-Methylheptan-3-one[b]	0·50	0·60	0·70
Cyclohex-1-en-3-ol	Cyclohexanone[b,e]	—	—	1·00
	Cyclohexanol[b,e]	—	—	1·00
Octan-4-ol	Octan-4-one[b]	0·40	0·70	0·80
Octan-3-ol	Octan-3-one[b]	0·80	1·20	1·20
Octan-2-ol	Octan-2-one[c]	1·60	3·20	3·60
Octan-1-ol	—[d]	—	—	—

[a] Incubations carried out with cell-free preparations of *P. oleovorans* and quantified by g.l.c. (see ref. 1). [b] Product identification based on comparisons of retention times and mass and n.m.r. spectral data of products isolated by preparative g.l.c. relative to authentic samples. [c] Identification based only on retention time comparisons with authentic samples. [d] No product detected by either g.l.c. or spectrophotometric observation of NAD^+ reduction with purified dehydrogenase samples. [e] Minor products were formed in trace amounts but were not further characterized.

Data obtained with cell-free preparations of *P. oleovorans*, which have been previously shown to be highly effective in the epoxidation of olefins on a preparative scale,[1] are shown in the Table. Thus, oct-1-en-3-ol gives primarily octan-3-one with a product yield approaching 1·5 mg ml^{-1}, comparable to the yield of 7,8-epoxyoct-1-ene from octa-1,7-diene under these conditions. Substitution at C-2 (*e.g.*, 2-methylhept-1-en-3-ol) diminishes reactivity, but yields of the saturated ketone still approach 0·5 mg ml^{-1}. It is apparent that carbon chain length also affects the reactivity of the alcohol substrates. This is similar to previous observations on the effect of carbon chain length in enzymatic epoxidation where activity decreases as the chain length decreases.[1] Linalool, which contains a tertiary allylic alcohol substituent, is non-reactive. We note that under the reaction conditions used, ketonization of saturated alcohols, potential intermediates in the reaction pathway from allylic alcohols, occurs readily, with octan-2-ol being the most reactive alcohol tested. It is apparent from the Table that reduction of the double bond can occur in the absence of alcohol dehydrogenation, *e.g.*, for oct-1-en-3-one or 2-methylhepten-3-one. For the allylic alcohol substrates, other products representing either alcohol dehydrogenation or double bond reduction are also observed. Thus, the data obtained with cell free preparations suggest that the easy production of ketones from allylic alcohols involves two independent processes; alcohol dehydrogenation and olefin reduction.

Partial purification of both the reductase and the alcohol dehydrogenase enzymes has been achieved. Multiple ion exchange and gel filtration column chromatography on the 30—60% ammonium sulphate fraction successfully resolved the dehydrogenase activity from the reductase activity. Neither enzymatic step requires O_2. Purified alcohol dehydrogenase showed a specificity for NAD^+, and, when incubated with oct-1-en-3-ol, produced only the unsaturated ketone. The partially purified reductase fraction, known to contain some alcohol dehydrogenase, requires NADH, and, when incubated with oct-1-en-3-ol, produced primarily the saturated alcohol along with a trace amount of octan-3-one. Thus, the alcohol dehydrogenation and the double bond reduction are two independent enzymatic reactions with all possible intermediates being observed when saturated ketones are produced from allylic alcohols. The substrate specificity of the alcohol dehydro-

genase reported here is highly unusual, and a number of synthetic applications which exploit its activity with the olefin reduction can be envisaged.

Laskin, Hou, and Patel (personal communication) report finding secondary alcohol-specific dehydrogenase activity in methanol-utilizing bacteria. The partially purified alcohol dehydrogenase obtained here did not catalyse octan-1-ol oxidation as measured by g.l.c. analyses or spectrophotometric NAD^+ reduction.

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