NOTES

Microbial Reduction of 1,3-Dioxo-2-Methyl-2-(3'-Oxo-6'-Carbomethoxyhexyl)-Cyclopentane to Form 1β-Hydroxy-3-Oxo-2β-Methyl-2α-(3'-Oxo-6'-Carbomethoxyhexyl)-Cyclopentane, an Intermediate for Steroid Total Synthesis

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The rate and extent of stereoselective reduction of 1,3-dioxo-2-methyl-2-(3'-oxo-6'-carbomethoxyhexyl)-cyclopentane to form the 1β-hydroxy-2β-methyl isomer by cultures of Schizosaccharomyces pombe ATCC 2476 was dramatically increased by addition to the fermentation of certain α,β-unsaturated ketones and allyl alcohol.

Investigations in our laboratory of the microbial reduction of 1,3-dioxo-2-methyl-2-(3'-oxo-6'-carbomethoxyhexyl)-cyclopentane (III) (Fig. 1) with growing cultures of Schizosaccharomyces pombe ATCC 2476, described in part elsewhere (R. P. Lanzilotta, U. S. Patent 3,793,148, 1974), have shown that the major product obtained was (−) 1β-hydroxy-3-oxo-2β-methyl-2α-(3'-oxo-6'-carbomethoxyhexyl)-cyclopentane (IVa) (Fig. 1), a valuable intermediate in the total synthesis of a variety of natural steroids (1). The major by-product formed was probably the 1β-hydroxy-2α-methyl isomer (IVb) (Fig. 1) (P. Bellet and T. Van Thuong, U.S. Patent 3,595,902, 1971).

During the course of these studies, it became evident that the rate and extent of reduction of III and the relative amounts of IVa and IVb formed varied, depending on the degree of purity of the starting material. Unexpectedly, the more highly purified samples of III gave the poorer conversions to IVa and the greater formation of by-products. It was, therefore, suspected that some substance present in the reaction mixture used in the synthesis of III (2), and therefore possibly present as an impurity in the recovered product, possessed the ability to enhance the rate and extent of conversion of III to IVa and reduce the amount of formation of the undesirable isomer IVb.

In fact, it was subsequently possible to demonstrate that 3-oxo-6-carbomethoxy-1-hexene (I) (Fig. 1), when added in low concentrations directly to fermentation reaction mixtures using purified III, greatly enhanced the rate and extent of formation of IVa and reduced the level of by-product formation (Table 1).

Moreover, it was possible to show that a variety of α,β-unsaturated ketones possessed this enhancing ability. The rather dramatic effect on yield of IVa obtained with some of the

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more active compounds examined is presented in Table 1. Names and structures of enhancing agents are given in Fig. 2. The fermentations were conducted in duplicate 300-ml conical flasks each containing 50 ml of nutrient broth (Difco) supplemented with 2% (wt/vol) glucose monohydrate. After inoculation with 3.5 ml of a 30-h liquid culture of S. pombe ATCC 2476, all flasks were incubated in an environmental rotary shaker (New Brunswick Scientific Co., New Brunswick, N.J.) at 300 rpm and maintained at 23 C. After approximately 18 h, each flask received 40 mg of III dissolved in 0.2 ml of acetone. The addition of III was repeated again at 5, 12, 24, 32, 48, and 56 h after the initial charge. In addition, all flasks received 0.6 ml of a supplemental glucose-nutrient broth solution (20 g of glucose·H₂O + 8 g of dehydrated nutrient broth [Difco] in 100 ml of water) with the last four additions of III.

Except for untreated controls, the various enhancing agents were added with each addition of the starting material (III). The concentrations of the enhancers are given in Table 1. Preliminary studies indicated that these concentrations are at or near the optimum. The progress of the fermentation was monitored by periodic sampling, followed by extraction with 1 volume of chloroform and thin-layer chromatographic analysis (Fig. 3). In all cases, incubations were terminated after 138 h. The contents of duplicate flasks were pooled and exhaustively extracted with chloroform. Portions of the chloroform extracts were then analyzed by high-pressure liquid chromatography with a refractive index detector.

In addition to the studies conducted with the unsaturated ketones, tests were conducted with several corresponding α,β-unsaturated alcohols, including 2-cyclohexene-1-ol, 3-buten-2-ol, and allyl alcohol (IX). The former two were without significant effect. However, allyl alcohol proved to be among the more effective enhancers, routinely resulting in more than threefold increases in formation of IVa (Table 1) with significant reduction in the formation of the undesirable isomer IVb. The thin-layer chromatographic analysis illustrated in Fig. 3 was typical of the results obtained with allyl alcohol. High-pressure liquid chromatographic analysis showed that the yield of IVa was 92 ± 3%.

Because of its availability, relative ease in handling, and comparative stability, allyl alcohol was chosen for further investigation, in which it was subsequently possible to demonstrate that the ability of low concentrations of allyl alcohol to enhance the conversion of III to IVa was not limited to S. pombe but could be demonstrated when the reductive transformation was carried out with, for example, cultures

Table 1. Effect of enhancers on conversion of III to IVa

| Enhancer | Amt added/addition (mg) | % Conversion with enhancer/% conversion without enhancer |
|----------|------------------------|----------------------------------------------------------|
| None     |                        |                                                          |
| I        | 0.75                   | 1                                                        |
| V        | 0.25                   | 3.0                                                      |
| VI       | 0.50                   | 2.7                                                      |
| VII      | 0.0625                 | 3.0                                                      |
| VIII     | 2.5                    | 4.2                                                      |
| IX       | 0.5                    | 4.4                                                      |

Fig. 2. Enhancer compounds.
FIG. 3. Thin-layer chromatogram of extracts of fermentation media samples taken after 138 h of incubation. (A) Untreated controls; (B) allyl alcohol-treated cultures. Solvent system was chloroform and acetone (80:20). Spots were visualized by spraying with 3% CeSO₄ in 3 N H₂SO₄ followed by heating. See Fig. 1 for identification of spots.

of Schizosaccharomyces malidevorans, Saccharomyces cerevisiae, and Saccharomyces uvarum.

The mechanism by which the various enhancers function has not as yet been determined. Possibly they act as inducers of the reductases involved or retard enzyme decay, thus increasing enzymatic half-life and prolonging the period of active reductive transformation.

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LITERATURE CITED

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