Carbohydrate Research, 252 (1994) 297–301
Elsevier Science B.V., Amsterdam

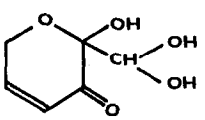
Note

A convenient laboratory procedure for the preparation of cortalcerone, a fungal antibiotic β-pyrone

Jiři Gabriel [a,*], Jindřich Volc [a], Elena Kubátová [a], Zdena Palková [b] and Martin Pospíšek [b]

[a] *Institute of Microbiology, Czech Academy of Sciences, Vídeňská 1083, CS-14220 Prague 4 (Czech Republic)*
[b] *Faculty of Science, Charles University, Viničná 5, 12843 Prague 2 (Czech Republic)*
(Received December 11th, 1992; accepted in revised form, July 15th, 1993)

The unsaturated β-pyrone antibiotic[1] cortalcerone (2-hydroxy-6*H*-3-pyrone-2-carboxaldehyde hydrate, **1**) was first prepared from aqueous macerates of mycelia from the basidiomycete *Corticium caeruleum*, by selective solubilization and precipitation followed by repeated silica-gel column chromatography. Subsequent studies[2,3] showed that cortalcerone is derived from D-glucose through a multi-step pathway involving oxidation of D-glucose to "D-glucosone" (D-*arabino*-2-hexosulose) and conversion of D-glucosone to cortalcerone by a mechanism involving a two-step dehydration by the new enzyme pyranosone dehydratase (aldos-2-ulose dehy-



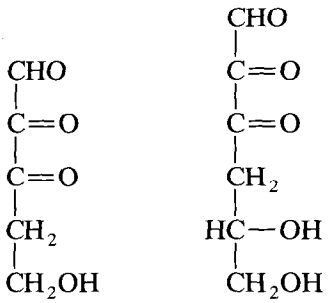
1

dratase). Dehydratases with the same activity on D-glucosone were later purified from another basidiomycetes, *Polyporus obtusus*[4] and *Phanerochaete chrysosporium*[5,6]. Recently, cortalcerone was prepared from D-*arabino*-hexos-2-ulose in 92% yield using purified aldos-2-ulose dehydratase from the basidiomycete *Polyporus obtusus*[7]. We now report a simple method for cortalcerone production based on the action of co-immobilized enzymes pyranose 2-oxidase, catalase, and aldos-2-ulose dehydratase on D-glucose followed by one-step chromatographic purification of the product. Furthermore, the immobilized enzyme preparation allows the

* Corresponding author.

SSDI 0008-6215(93)E0244-U

conversion of D-xylose and 6-deoxy-D-glucose to 5-hydroxy-2,3-dioxopentanal (**2**) and D-*glycero*-5-hydroxy-2,3-dioxohexanal (**3**), respectively[6].



2 **3**

Besides aldos-2-ulose dehydratase, pyranose oxidase (EC 1.1.3.10) is also produced by *Phanerochaete chrysosporium*. This enzyme was co-immobilized, together with aldos-2-ulose dehydratase and bovine catalase, on cell-wall fragments of the producing fungus by cross-linking with glutaraldehyde. Cortalcerone was then prepared directly from D-glucose by a simple incubation of immobilized preparation with a 2% solution of D-glucose. The activity of the immobilized preparation, measured as the activity of aldos-2-ulose dehydratase, showed good stability on

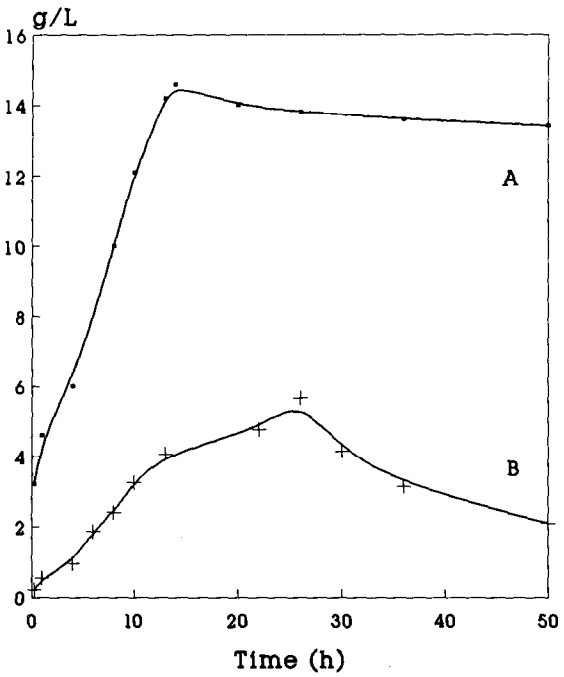


Fig. 1. Production curves of cortalcerone by the immobilized enzyme preparation (A) and by the washed intact mycelium of the basidiomycete *Phanerochaete chrysosporium* (B).

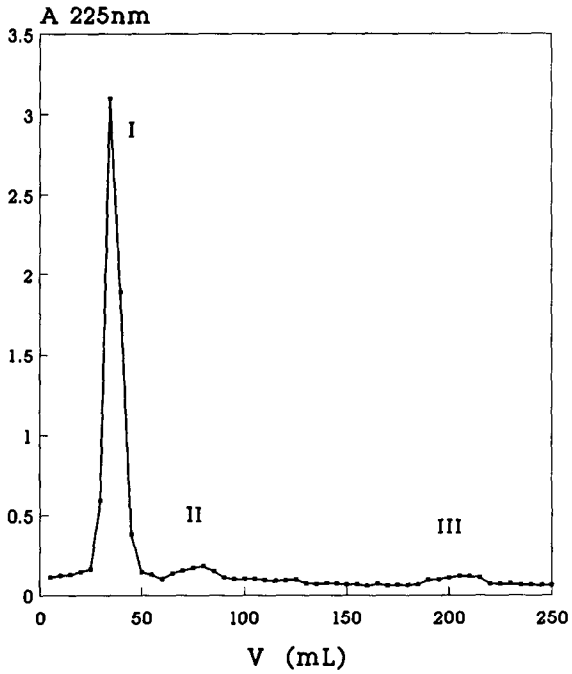


Fig. 2. Preparative chromatogram of (I) cortalcerone, (II) 2-furylglyoxal, and (III) sugar intermediates on Dowex 50 in the Ca^{2+} form. Mobile phase distilled water, UV detection at 225 nm.

storage at 4°C, decreasing to ca. 80% after 1 month and then remaining stable for another 2 months. In comparison with the active mycelium, the enzymic preparation gave higher yields of cortalcerone (Fig. 1).

In aqueous solution, cortalcerone spontaneously recyclizes to 2-furylglyoxal. This process is both temperature- and pH-dependent; solutions in acetate buffer of pH 5.0 or in distilled water, retained more than 95% of the initial concentration of cortalcerone after 3 days at 4°C and ca. 80% at room temperature. In the pH range 7.5–9.0, cortalcerone concentration decreased to less than 70% of the initial concentration in 24 h under laboratory conditions. The formation of 2-furylglyoxal in both acidic and alkaline solutions was confirmed by TLC.

Purification of cortalcerone was carried out on a column packed with Dowex 50 resin in the Ca^{2+} form[8]. The use of water as the mobile phase gave a good separation of cortalcerone from its degradation product 2-furylglyoxal and sugar intermediates (Fig. 2). Moreover, purified cortalcerone could be immediately lyophilized. The total yield of cortalcerone was ~ 90%; e.g., an amount of 63.4 mg of lyophilized product of enzyme transformation sampled on the column yielded 54.4 mg (86%) of pure cortalcerone and 6.1 mg of 2-furylglyoxal. The purity of the final product was confirmed by HPLC (Fig. 3).

Cortalcerone was reported to exhibit antimicrobial activity on *Escherichia coli, Bacillus subtilis, Serratia marcescens, Staphylococcus aureus*[7], and *S. pyogenes*[1]. In

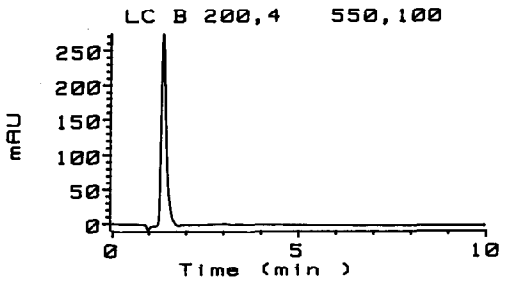


Fig. 3. HPLC chromatogram of cortalcerone on a C_{18} column (Hewlett–Packard, 100×4.6 mm) eluted with aqueous acetonitrile (70%, pH 5.00). UV detection at 200 nm.

our quantitative tests this compound inhibited the growth of *E. coli* [minimal inhibitory concentration (MIC) 480 mg/L] and *B. subtilis* (MIC 260 mg/L), but no effect on yeast (*Saccharomyces cerevisiae, Candida tropicalis, Cryptococcus albidus*, or *Trichosporon cutaneum*) was observed.

5-Hydroxy-2,3-dioxopentanal and D-*glycero*-5-hydroxy-2,3-dioxohexanal[6] could also be produced by the action of the immobilized enzyme preparation on D-xylose and 6-deoxy-D-glucose, respectively. Since proper analytical methods for the detection of tricarbonyl sugars have not been published yet, production of the compounds was confirmed by converting them into their *N,N*-diphenylhydrazones[6]. The above tricarbonyl sugars were produced as mixtures with the corresponding intermediate aldosuloses in ca. 40% yields.

EXPERIMENTAL

Enzyme immobilization.—The fungus was grown as previously described[5,6]. An 11-day old mycelium containing both pyranose oxidase (EC 1.1.3.10) and pyranosone dehydratase (EC not yet assigned) enzymes, was used. Washed mycelium (75 g wet weight) was suspended in distilled water (120 mL) containing catalase (EC 1.11.1.6, Reanal, Hungary, 10 mL) and homogenized (5 × 1 min, Ultra-Turrax, max speed) on ice. To the resulting suspension Sedipure (BASF, Germany, 0.6 g) was added, pH was adjusted to 7.8 with NaOH (0.5 M) and glutaraldehyde (50%, 2.4 mL) was added in drops under vigorous stirring. This preparation was quickly frozen in a thin layer and held overnight at −20°C. After melting, residual glutaraldehyde and other soluble compounds were removed by decantation in distilled water. The immobilized preparation was stored as a suspension in water at 4°C.

Preparation of cortalcerone.—To a solution of D-glucose (5 g in 250 mL of water) in Erlenmeyer flasks, the immobilized enzymic preparation (10 g wet weight) was added and the mixture was incubated at 25°C with agitation (reciprocal frequency of 2 Hz). After 14 h, when the cortalcerone concentration, calculated from absorbance at 225 nm reached its maximum (A, Fig. 1.), the enzymic

preparation was filtered and the resulting filtrate was lyophilized. Final chromatographic purification of cortalcerone was carried out on a Dowex 50 column (250 × 20 mm i.d.) in the Ca^{2+} form[8]. Mobile phase consisted of distilled water (2 mL/min). Cortalcerone was detected by monitoring the eluate at 225 nm. The yield of amorphous cortalcerone was ~ 4.3 g (86%).

Analytical methods.—Aldos-2-ulose dehydratase and pyranose oxidase activities were measured as previously described[5,6]. Cortalcerone was determined by either a HPLC method[9] or from its absorbance at 225 nm and log ϵ_{225} (1,4-dioxane, 20°C) 3.9 (Baute et al.[1]). Aldoses and aldosuloses were characterized by TLC on cellulose-coated sheets with DPA-reagent as previously reported[10]. Tricarbonyl sugars were detected by TLC on silica gel sheets as their *N*,*N*-diphenylhydrazones, as previously reported[10].

Antibiotic assay.—The determination of MIC values was performed by the agar diffusion method on 8-mm thick, 60 × 60 cm agar plates inoculated with microorganisms (*E. coli, B. subtilis, S. cerevisiae, C. tropicalis, C. albidus* and *T. cutaneum*). Aliquots (100 μL) of cortalcerone were tested at concentrations of 2000, 1000, 500, 250, 125, 62.5, 31.3, 15.6, 7.8, and 3.9 mg/L, respectively. The MIC values were calculated from the linear dependence of diameters of inhibition zones (appearing after 24 h incubation at 28°C) on log concentration of the compound.

REFERENCES

1 R. Baute, M.A. Baute, G. Deffieux, and M.J. Filleau, *Phytochemistry*, 15 (1976) 1753–1755.
2 M.A. Baute, R. Baute, G. Deffieux, and M.J. Filleau, *Phytochemistry*, 16 (1977) 1895–1897.
3 R. Baute, M.A. Baute, and G. Deffieux, *Phytochemistry*, 26 (1987) 1395–1397.
4 K.E. Koths, R.F. Halenbeck, and P.M. Fernandez, US Pat., 4569910 (1986), *Chem. Abstr.*, 104: P184888 (1986).
5 J. Volc, E. Kubátová, P. Sedmera, G. Daniel, and J. Gabriel, *Arch. Microbiol.*, 156 (1991) 297–301.
6 J. Gabriel, J. Volc, P. Sedmera, G. Daniel, and E. Kubátová, *Arch. Microbiol.*, 160 (1993) 27–34.
7 K. Koths, R. Halenbeck, and M. Moreland, *Carbohydr. Res.*, 232 (1992) 59–75.
8 J. Gabriel, J. Volc, E. Kubátová, and M. Musílek, Czech. Pat. 276695 (1992).
9 J. Gabriel, O. Vacek, E. Kubátová, and J. Volc, *J. Chromatogr.*, 542 (1991) 200–203.
10 K.-E. Eriksson, B. Pettersson, J. Volc, and V. Musílek, *Appl. Microbiol. Biotechnol.*, 23 (1986) 257–262.