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Short Communication

High-performance liquid chromatographic determination of the antibiotic cortalcerone

JIŘÍ GABRIEL*, OLDŘICH VACEK, ELENA KUBÁTOVÁ and JINDŘICH VOLC
Institute of Microbiology, Czechoslovak Academy of Sciences, Vídeňská 1083, 142 20 Prague 4 (Czechoslovakia)

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ABSTRACT

Cortalcerone (2-hydroxy-6*H*-3-pyrone-2-carboxaldehyde hydrate) is an antibacterial antibiotic produced by a number of basidiomycetous fungi. Its biosynthesis is connected with C-2 oxidation of D-glucose. A method for the reliable determination of this antibiotic is required for the study of the mechanism of its biosynthesis and for the determination of its pharmacological properties. The high-performance liquid chromatographic determination of cortalcerone in the presence of D-glucose, D-fructose, D-*arabino*-2-hexosulose, D-gluconic acid and D-*arabino*-2-hexulosonic acid on an amino-phase column with 70% aqueous acetonitrile (pH 5.00) and UV detection at 195 nm is reported. A linear relationship between response and cortalcerone concentration was found in the range 0.25–10.00 mg/ml. The detection limit is 6.5 μg/ml.

INTRODUCTION

Cortalcerone, an antibacterial antibiotic produced by a number of basidiomycetous fungi [1,2], is a β-pyrone compound unusual in nature, 2-hydroxy-6*H*-3-pyrone-2-carboxaldehyde hydrate (Fig. 1). Its biosynthesis starts from D-glucose and consists of two enzymatic steps; in the first step; *D*-glucose is oxidized by the action of the enzyme pyranose 2-oxidase into D-*arabino*-2-hexosulose (D-glucosone) [3], which is then dehydrated to cortalcerone by a new enzyme, pyranosone dehydratase [4] via putative unstable intermediates that have not yet been identified.

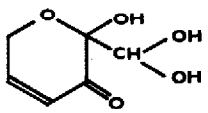

Fig. 1. Structure of cortalcerone.

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A method for the reliable determination of cortalcerone is required for the study of the mechanism of its biosynthesis. High-performance liquid chromatography (HPLC) on silica [5], ion-exchange [6], reversed-phase [7] or amino-phase [8] columns has often been used for the determination of sugars and/or their derivatives in natural samples. The separation of sugars on various stationary phases has been reviewed and discussed in detail [9,10]. For cortalcerone, only a qualitative thin-layer chromatographic assay is available [2].

A simple HPLC method has been devised for the determination of cortalcerone in the presence of D-glucose, D-fructose, D-*arabino*-2-hexosulose, D-gluconic acid and D-*arabino*-2-hexulosonic acid using an amino-phase column and UV photometric detection. The method was used to examine the time course of cortalcerone production by the washed intact mycelium of the basidiomycete *Phanerochaete chrysosporium* during incubation with a solution of pure D-glucose.

EXPERIMENTAL

Chemicals

Cortalcerone was isolated [11] from submerged cultures of the basidiomycete *Phanerochaete chrysosporium* (CCBAS 571, Culture Collection of Basidiomycetes, Institute of Microbiology, Prague (Czechoslovakia) by a procedure described by Baute *et al.* [1]. D-*arabino*-2-hexosulose was synthesized enzymatically [12] from D-glucose. D-Glucose was obtained from Fluka (Buchs, Switzerland), D-fructose was of analytical-reagent grade from Lachema (Brno, Czechoslovakia), sodium D-gluconate was purchased from Koch-Light Labs. (Haverhill, U.K.) and calcium D-*arabino*-2-hexulosonate from Merck (Darmstadt, Germany).

Sample preparation

All standards were prepared as aqueous solutions of concentration 10 mg/ml unless noted otherwise. The time course of cortalcerone production was studied as follows: 10 g of washed intact mycelium of the basidiomycete *Phanerochaete chrysosporium* were suspended in 50 ml of a 2% aqueous solution of D-glucose. The suspension was shaken at 20°C for 48 h, than 100-μl samples of liquid were taken and, after filtration through an Acrodisc 13 disposable filter unit (Gelman Sciences, Ann Arbor, MI, U.S.A.), 10-μl aliquots were chromatographed.

Column liquid chromatography

An HP 1090 liquid chromatograph with a diode-array detector (Hewlett-Packard, Amstelveen, The Netherlands) was used. For quantitative purposes compounds were detected at 195 nm. The separation was performed on an analytical column (200 mm × 4 mm I.D.) packed with Separon SGX NH_2, 7 μm (Tessek, Prague, Czechoslovakia). The mobile phase was acetonitrile–water (70:30) of pH 5.0 adjusted by addition of 0.1 *M* phosphate buffer (1 ml of buffer to 1 l of the mobile phase), at a flow-rate of 1.5 ml/min. Quantification was performed by recording the ratio of the peak area to that of calibration standards of cortalcerone injected directly into the HPLC column.

Precision and reproducibility studies

The precision and linearity were validated by analysing ten calibration standards containing 0–10.00 mg/ml of cortalcerone in triplicate over 48 h. The reproducibility of the peak-area ratios over the calibration range, expressed as relative standard deviation (R.S.D.), ranged from 5.2 to 7.0%. The R.S.D. of the peak-area ratio was determined at each concentration level of the calibration graph. The slope and the correlation coefficient of the calibration graphs were also calculated.

RESULTS AND DISCUSSION

Cortalcerone is a relatively unstable compound that recyclizes spontaneously to 2-furylglyoxal. In the pH range 5.0–7.0 cortalcerone forms an equilibrium mixture with 2-furylglyoxal which contains 85.6% of cortalcerone. These solutions are stable for 2 days under laboratory conditions. The heat lability of cortalcerone precludes the use of ion-exchange columns which are usually operated at elevated temperatures. For this reason and the earlier successful separation of D-*arabino*-2-hexosulose on an amino-bonded column [8], this type of stationary phase was chosen. The formation of Schiff bases of carbonyl compounds with the column packing [13] can be overcome by using a mobile phase buffered on the acidic side.

Dicarbonyl sugars in solution possess a number of tautomeric forms [14,15]. To check possible complications resulting from different chromatographic behaviours of different forms of D-*arabino*-2-hexosulose, we sampled aliquots of solutions prepared in 0.1 *M* acetate buffers of pH 4.0, 4.5, 5.0, 5.5, 6.0 and 6.5 after equilibration for 2 h. Comparison of the chromatograms showed that negligible changes in the retention time and peak area of D-*arabino*-2-hexosulose occurred. As D-*arabino*-2-hexosulose decomposes in the presence of trace amounts of a base [16], the mobile phase should be buffered on the acidic side.

D-Glucose and D-*arabino*-2-hexosulose coelute when the mobile phase is not buffered or when phosphate buffer of pH 6.0 is used. Considering the stability of cortalcerone and D-*arabino*-2-hexosulose, a mobile phase of pH 5.0 was adopted. In the concentration region between 60 and 80% of acetonitrile in the mobile phase, the best resolutions of both D-glucose–D-*arabino*-2-hexosulose and cortalcerone–2-furyl-

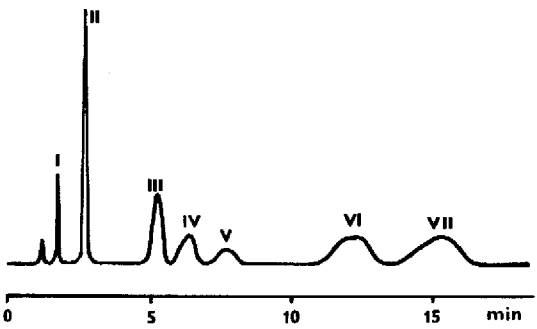

Fig. 2. Chromatogram of (I) 2-furylglyoxal, (II) cortalcerone, (III) D-fructose, (IV) D-glucose, (V) D-*arabino*-2-hexosulose, (VI) D-*arabino*-2-hexulosonic acid and (VII) D-gluconic acid using UV detection at 195 nm.

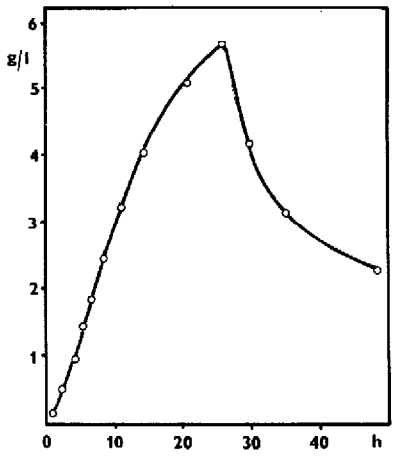

Fig. 3. Production curve of cortalcerone by the washed intact mycelium of the basidiomycete *Phanerochaete chrysosporium*.

glyoxal were obtained when 70% of acetonitrile of pH 5.0 was used; reliable measurements were then achieved.

Fig. 2 shows the measurement of cortalcerone using UV detection at 195 nm in the presence of D-glucose, D-fructose, D-*arabino*-2-hexosulose, D-gluconic acid and D-*arabino*-2-hexulosonic acid. These compounds could play a role in the biochemical oxidation of D-glucose in fungi. A linear relationship was found between the peak area at 195 nm and the cortalcerone concentration in the range 0.25–10.00 mg/ml (slope 0.267 absorbance/μg, correlation coefficient 0.9995, R.S.D. 6.3% from five parallel determinations). At a signal-to-noise ratio of 3, the detection limit was found to be 6.5 μg/ml.

The method was used to determine the time course of cortalcerone production by the washed intact mycelium of the basidiomycete *Phanerochaete chrysosporium*. The production curve is shown in Fig. 3. The maximum cortalcerone concentration was found after incubation for 26 h.

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