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CONVERSION OF GLUCOSE TO CORTALCERONE VIA GLUCOSONE BY *CORTICIUM CAERULEUM*

MARIE-ANTOINETTE BAUTE*, ROBERT BAUTE*†, GÉRARD DEFFIEUX‡ and MARIE-JOSÉ FILLEAU‡

*Laboratoire de Cryptogamie et Microbiologie industrielle; ‡Laboratoire de Chimie analytique, Faculté de Pharmacie, Université de Bordeaux II, Rue Leyteire, 33000 Bordeaux, France

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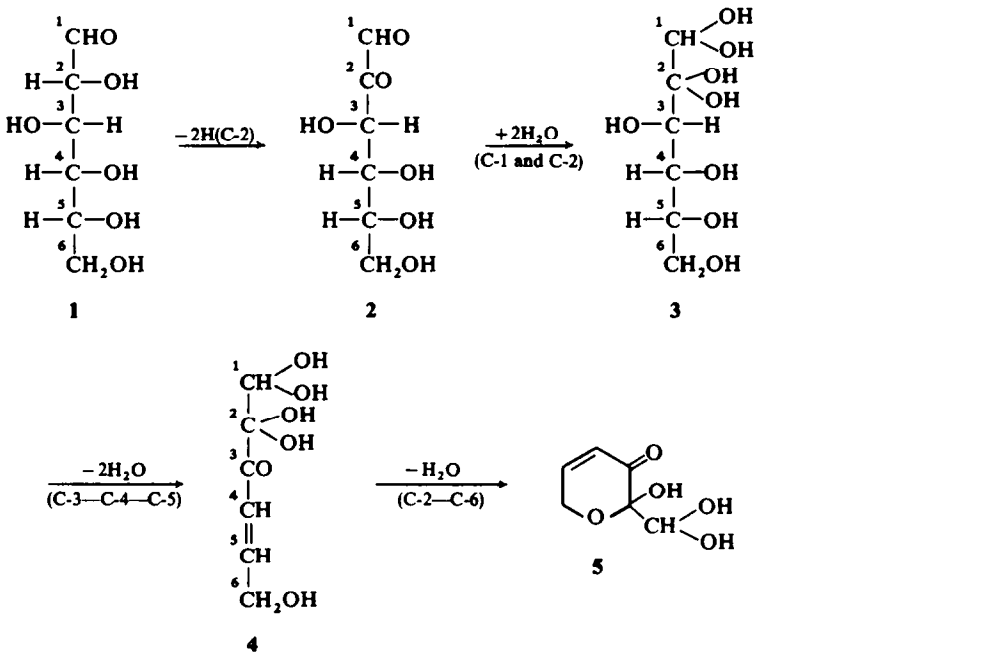
Abstract—Glucose was the sole compound to be converted to cortalcerone by 'activated' blue mycelia of *Corticium caeruleum*; the first step of this transformation was shown to be the oxidation of glucose to glucosone. The conversion of glucosone to cortalcerone implies a novel enzymatic dehydrating activity which could be specific for *C. caeruleum*.

INTRODUCTION

In a previous publication [1] we reported the identification of cortalcerone (**5**, Scheme 1) an antibacterial compound produced by the fungus *Corticium caeruleum* when its blue mycelium is macerated in water in the presence of 'activating agents' (supraoptimal temperature, vapours of organic solvents, toluene).

In our early studies, we noticed that enrichment of the liquid with glucose resulted in an almost quantitative increase in cortalcerone production. That glucose could serve as a precursor of this compound was not surprising, considering the structure of cortalcerone; the overall conversion of the former ($C_6H_{12}O_6$) to the latter ($C_6H_8O_5$) would then involve both an oxidation and a dehydration. We have also pointed out the resemblance between cortalcerone and unsaturated osones [1]. These facts were consistent with Scheme 1, in which cortalcerone is the stable cyclic form of an unsaturated intermediate arising from glucosone through a double dehydration. Molecular models show that the ultimate, non-enzymatic cyclization (i) would require a

†To whom correspondence should be addressed.



Scheme 1. Hypothetical pathway of conversion of glucose (**1**) to cortalcerone (**5**) via glucosone (**2**). The bi-hydrated form (**3**) of glucosone could be enzymatically dehydrated twice to give the unstable unsaturated intermediate (**4**) which would spontaneously cyclize to yield racemic cortalcerone (**5**).

cis-configuration of the double bond, and (ii) might occur on either of both OH at C-2, thus giving racemic cortalcerone—which is actually obtained [1].

In the present publication, we show (i) that glucose is the specific precursor of cortalcerone, and (ii) that its conversion to the latter does proceed via glucosone.

RESULTS

Glucose as the specific precursor of cortalcerone

Only glucose, or glucose derivatives hydrolysable by *Corticium caeruleum* (maltose, trehalose, soluble starch), proved capable of increasing cortalcerone production. This additional production (an average of 0.53 mM per mycelium) was practically equal to the extra quantity (0.55 mM) of glucose in the medium (as such or released by hydrolysis). With all other compounds, no significant increase over the normal yield (0.25 mM) based on endogenous glucose (derived from glycogen) was observed. Negative results were thus recorded for sorbose, galactose, 2-deoxyglucose, sucrose, raffinose, fructose, mannose, xylose, arabinose, mannitol and sorbitol. Lactose exhibited intermediate behaviour, yielding an extra production of 0.22 mM of cortalcerone; its ineffectiveness as a carbon source suggests that it was only partially hydrolyzed.

Glucosone as an intermediate in this conversion

That glucosone is an intermediate is supported by several experimental results: (i) oxygen appeared to be necessary for the *overall* conversion of glucose to cortalcerone, which was not observed under nitrogen; (ii) aerobic activation of mycelia in the presence of glucosone resulted in the same increase (0.25 to 0.79 mM) of cortalcerone production as if glucose had been added at an equal concentration; (iii) nitrogen did not prevent glucosone from being converted to cortalcerone by the fungus; the lower production (0.52 mM compared to 0.79 mM) is easily explained by the non-conversion of endogenous glucose under these conditions; and (iv) attempts to trap glucosone in aqueous macerates of 'conventionally activated' mycelia [1] were unsuccessful, but this compound did accumulate when activation (i.e. maceration of mycelia in aqueous glucose solution added with toluene) was carried out on mycelia which had been previously shaken aerobically for 24 hr *without toluene*. Under these conditions—to which we will refer as 'delayed activation'—no cortalcerone was formed, but glucosone could be trapped by adding phenylhydrazine to the macerates; the resulting yellow precipitate was identified as glucosazone (see Experimental). The formation of glucosazone at room temperature is typical of glucosone, and excludes the intervention of glucose, which yields the osazone only on heating [2]. We also observed the almost instantaneous formation of 'glucose quinoxaline' by adding *o*-phenylenediamine to the macerates [2].

*Thawing of previously frozen mycelia proved to be an excellent activating treatment.

DISCUSSION

Osones, $HOH_2C—(CHOH)_n—CO—CHO$, are known principally as arising through hydrolysis of osazones, but a few have been shown to occur naturally arising from the enzymatic oxidation of the corresponding sugar. Thus, sorbose is converted to sorbosone by the bacteria *Acetobacter suboxydans* var. *5-ketogluconicum* [3] and *Gluconobacter melanogenus* [4]; glucosone is produced from glucose by the marine red alga *Iridococcus flaccidum* [5], by the mollusc *Saxidomus giganteus* [6], and by the fungi *Aspergillus flavus* [2, 7] and *Polyporus obtusus* [8, 9]. From the latter, the enzyme concerned (pyranoseoxidase) was isolated and shown to oxidize glucose in the presence of oxygen. Thus, the oxidation of glucose to glucosone by *Corticium caeruleum* constitutes, as far as we know, the seventh such example, and the third to be found in a fungus. *C. caeruleum* is, however, unique in its ability to convert glucosone to cortalcerone, and nothing is as yet known regarding this further enzymatic transformation.

EXPERIMENTAL

Effects of various compounds on cortalcerone production. Blue mycelia of *C. caeruleum*, grown as previously described [1], were stored frozen* until needed, then coarsely ground in H_2O (10 ml per mycelium) and, after a × 10 dilution, the suspension was distributed in 250 ml flasks (100 ml per flask, this vol. corresponding to one mycelium averaging a dry wt of 100 mg). Glucose and other hexoses were added in the ratio of 100 mg (0.55 mM) to one mycelium. Concns of sugars were calculated so that they would liberate on hydrolysis the same amount of glucose as above. For pentoses and sugar alcohols, the carbon ratio was taken into account. Aerobic testings were carried out by incubating the open flasks for 24 hr at room temp. on a rotary shaker (150 rev./min); to realize anaerobic conditions, nitrogen was bubbled through the suspension for an equal time.

Assay of cortalcerone. Homologous mycelial suspensions were pooled and filtered. From their optical densities at 230 nm [1], corresponding molar concns of cortalcerone were deduced, using a standard curve obtained from known aq. concns of the crystalline compound.

'Delayed activation' of mycelia. Entire mycelia were separated from their agar substratum. Each of them was introduced into a 250 ml flask containing 100 ml H_2O and shaken for 24 hr at room temp. at 150 rev./min. Then each flask was supplied with 1 ml of toluene and 1 ml of a 10% aq. glucose soln and shaken again for 24 hr. Combined aq. macerates were then tested for cortalcerone and glucosone.

Preparation of reference glucosone. Glucosone was prepared synthetically and identified according to [10] and [2].

Identification of free glucosone in mycelial macerates after delayed activation. (a) *Formation of glucosazone at room temp.* To 10 ml of the macerate were added successively 1 ml of HOAc and 1 ml of phenylhydrazine. A yellowish-brown ppt. appeared immediately, which was collected by filtration, washed with 10% HOAc, H_2O, dried and dissolved in EtOH. Isolation of glucosone from pooled solns was carried out by PLC (Si gel, $CHCl_3$–MeOH, 91:9): several phenylhydrazones separated quickly from the bright yellow streak of glucosazone which practically did not migrate. From the combined ethanolic eluates glucosazone was re-crystallized: its mp (209°) and IR spectrum were identical with those of authentic glucosazone. (b) *Formation of 'glucose quinoxaline', or 2-(tetrahydroxy-*n*-butyl) quinoxaline.* The macerate was first concd × 2 under red. pres. at a temp. not exceeding 40°. To 10 ml of the concentrate were added successively 0.50 g *o*-phenylenediamine dissolved in the minimal quantity of cold H_2O and 1 ml 10% NaOH. After vigorous shaking for a few min, a ppt. began to separate which was rapidly collected by filtration. Recrystallization from EtOH yielded a white crystalline product. Mp 193–194° was not depressed by

admixture with glucose quinoxaline obtained from synthetic glucosone. IR spectra of both compounds were identical. Glucose did not yield the quinoxaline under similar experimental conditions.

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