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PROPOSED PATHWAY TO THE PYRONES CORTALCERONE AND MICROTHECIN IN FUNGI

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Key Word Index—*Corticium caeruleum*; *Pulcherricium caeruleum*; *Morchella costata*; fungi; morels; cortalcerone; microthecin; pyrones; osones; 1,5-anhydro-D-fructose; carbohydrates; dehydratase; bioconversion.

Abstract—Carbonyl forms of D-glucopyranosone, 1,5-anhydro-D-fructopyranose and D-xylopyranosone possess a three-carbon steric arrangement which could be acted on by the dehydratase(s) of the cortalcerone or microthecin-producing fungi. With the first two substrates, two dehydrations via a spontaneously recyclized straight-chain intermediate would yield racemic cortalcerone or microthecin; D-xylosone would undergo a single dehydration yielding a compound for which a structure is proposed.

INTRODUCTION

From our early observations on the production of the antibiotic pyrone cortalcerone (Scheme 1, **6**, R = OH) from D-glucose via D-glucosone (D-arabino-2-hexosulose) by mycelia of the lignicolous fungus *Corticium caeruleum* subjected to plasmolytic treatments (e.g. freezing–thawing), we deduced a hypothetical pathway in which a straight-chain form of D-glucosone undergoes two enzymatic dehydrations giving an unstable, unsaturated intermediate that yields racemic cortalcerone on spontaneous cyclization [1]. More recently [2], we have shown that microthecin (**6**, R = H), the alcohol homologue of cortalcerone in morels and some other fungi [3], arises from 1,5-D-anhydrofructose. This finding, together with further experimental results, now enables us to propose a more likely pathway for the biogenesis of both pyrones, as well as for the enzyme-catalysed transformation of a third substrate, D-xylosone.

RESULTS

Any proposed pathway should be consistent with the following experimental results. (1) Cortalcerone and microthecin were isolated from the producing fungi as racemic compounds [3, 4]. (2) Spectrophotometric study of the enzymatic formation of cortalcerone from D-glucosone, or of microthecin from 1,5-D-anhydrofructose, showed that the appearance of their characteristic absorption peak at 230 nm was preceded by an early A_{max} at 265 nm that gradually gave place to the former (see Experimental). (3) In a previous investigation [5] of the action of *C. caeruleum* enzyme on several sugars (none was acted on) and osones (D-glucosone, D-galactosone, L-sorbosone, D-arabinosone, L-arabinosone, D-xylosone), we observed that, with the exception of D-glucosone, the enzyme was active only upon D-xylosone (D-threo-2-pentosulose) (but not upon D-xylose). We inferred that the substrate of the enzyme should be an osone in which the hydroxyl groups at C-3 and C-4 should have the same configuration as in D-glucose, and, if a hexosone, should have an additional D-configuration for the OH at C-5. Because 1,5-D-anhydrofructose, which is not an osone, is also a substrate, these conclusions should be at least partially revised. (4) The action of the *C. caeruleum* enzyme on D-xylosone resulted in an early absorption at 265 nm which disappeared gradually, as observed with D-glucosone and 1,5-D-anhydrofructose, but was not replaced by another UV or visible absorption. (5) Semi-purified enzymatic extracts from *C. caeruleum* and *Morchella costata* were active on either D-glucosone, 1,5-D-anhydrofructose or D-xylosone.

A pathway (Scheme 1, left) in which the enzymes act upon the carbonyl forms of 1,5-D-anhydrofructopyranose (**1**, R = H) and D-glucopyranosone (**1**, R = OH) is in accordance with all results. The dehydratase presumably acts on the steric arrangement at positions 2–4 (**1,5,10**) causing dehydration with removal of the OH from C-4; the resulting ketoenol **2** would account for the transient absorption at 265 nm, as shown by Woodward rules. The strain due to the double bond should cause the ring to open (**3**) and the tautomeric intermediate **4** would spontaneously recyclize between C-6 and the hydrated carbonyl at position 2, giving the non-absorbing, racemic compound **5**, which itself displays the arrangement required by the dehydratase; a second enzymatic dehydration would then yield cortalcerone or microthecin (**6**) with a concomitant increase in the absorption at 230 nm. A similar mechanism (Scheme 2) would apply to D-xylosone (**10**), except that the compound derived from spontaneous recyclization of **13**, and for which the non-absorbing structure **14** may be anticipated, cannot be dehydrated by the enzyme and therefore is the end-product of the pathway.

Further points can be discussed. (1) The intermediate **13**

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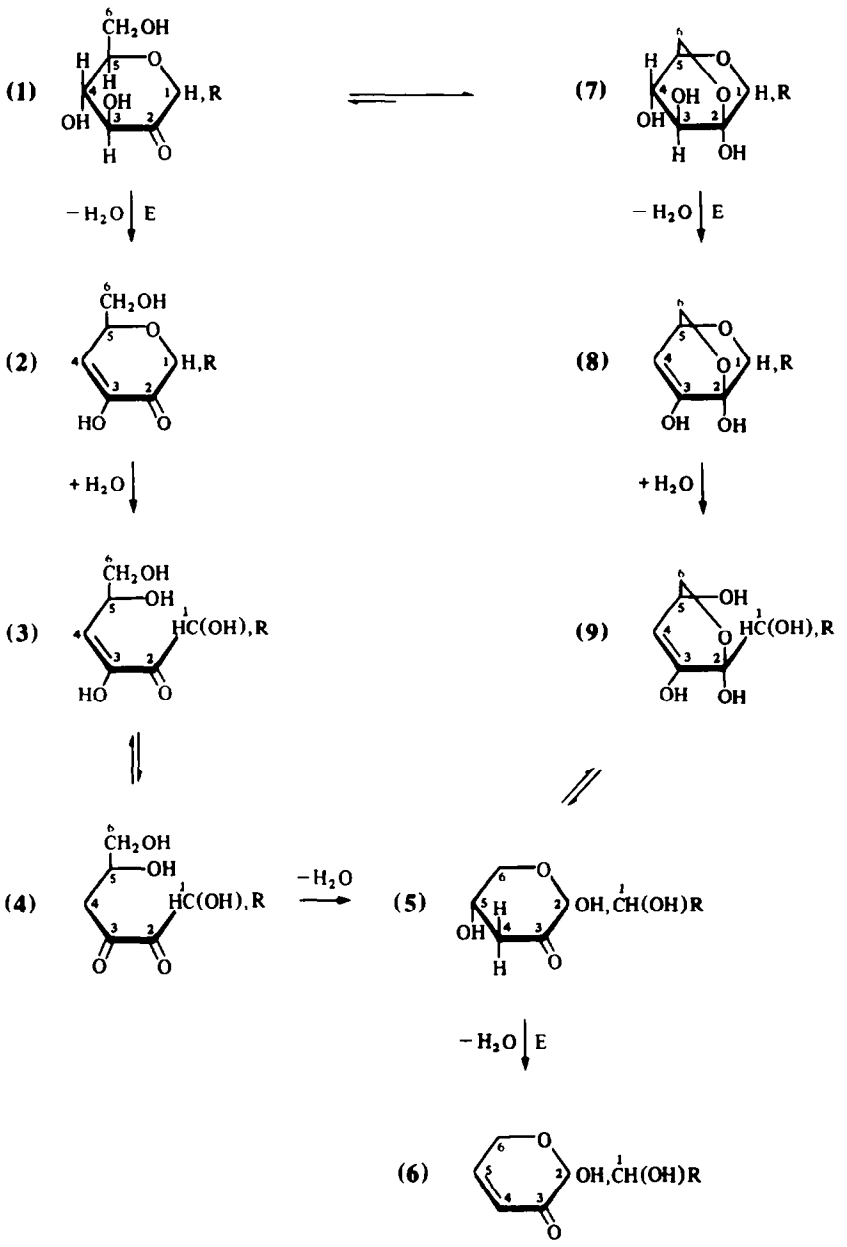
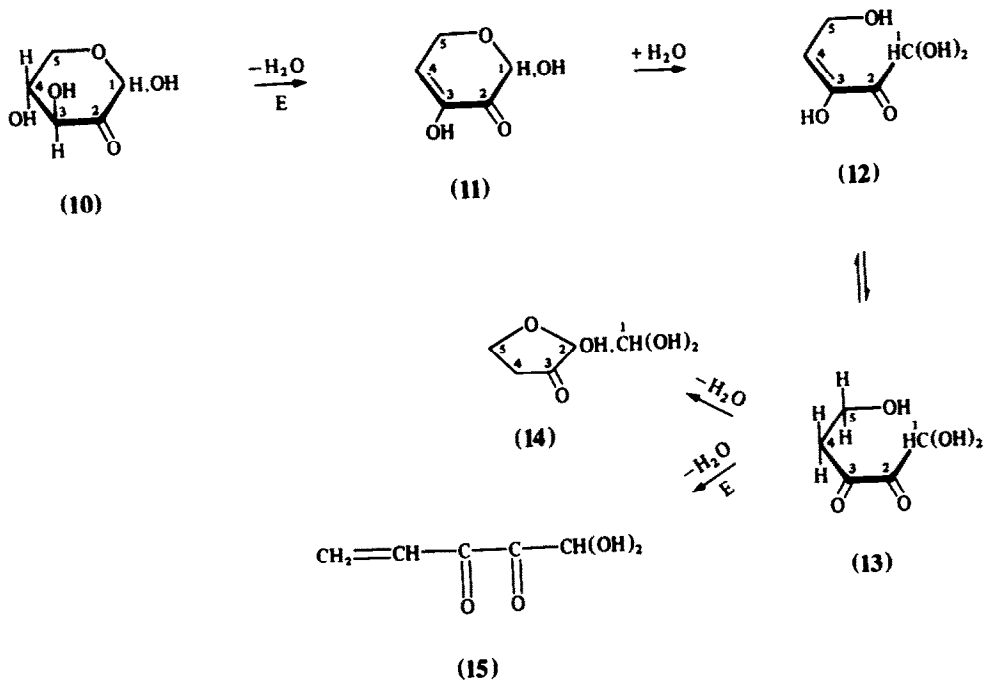


Scheme 1. Possible routes for the bioconversions of D-glucosone (1, R = OH) to cortalcerone (6, R = OH) and of 1,5-D-anhydrofructose (1, R = H) to microthecin (6, R = H). E: enzymatic dehydrations. The most likely pathway is via intermediates 2,3,4 (see text).

possesses at positions 3,4,5 an arrangement which, in theory, might be acted on by the dehydratase. Such a reaction would prevent further recyclization and yield the highly conjugated compound **15** which would exhibit a strong absorption in the visible range. Thus, a pyranose ring (or perhaps only a cyclohexane one?) seems to be required for enzyme action—which, by the way, precludes enzymatic dehydration of intermediate **4**, followed by spontaneous recyclization. (2) We have previously reported that, in aqueous solution, the equilibrium between the carbonyl and the bicyclic form of 1,5-D-anhydrofructose (1 and 7, R = H) is presumably largely in favour of the latter, since no carbonyl group was detected by ^{13}C NMR [2]; however, action of the enzyme on this bicyclic form seems to be precluded, since this route (Scheme 1, right), via intermediates **8** and **9**, would explain neither the transitory absorption at 265 nm nor the racemic condition of C-2 in microthecin (in the absence of ring-opening and recyclization). Therefore, it is much more likely that the enzyme acts on the carbonyl form, shifting the equilibrium to it (a similar conclusion probably applies to D-glucosone). Other evidence is afforded by the fact that the enzyme acts on D-xylosone, which has no bicyclic form, and not on D-xylose, in which the arrange-



Scheme 2. Pathway of the enzymatic dehydration (E) of D-xylosone (10) to the likely compound 14; further enzymatic dehydration of 13 yielding compound 15 has to be excluded (see text).

ment at positions 2,3,4 is the same as in 7.

We have pointed out on several occasions the similarity of action of the partially purified enzymatic extracts of *C. caeruleum* and of morels upon D-glucosone, 1,5-D-anhydrofructose and D-xylosone. Electrophoretic studies of more purified extracts should indicate whether these extracts contain a single type of non-specific dehydratases, since they are unaffected by the presence or the absence of an OH at C-1 and a CH_2OH at C-5, or a mixture of more specific enzymes.

EXPERIMENTAL

Semi-purified enzyme extracts from Corticium caeruleum *and* Morchella costata were prepared as previously described [2].

Action of M. costata *extract on osones* was studied as reported for *Corticium caeruleum* [5].

Transient absorption at 265 nm. An aq. dilution of the semi-purified enzymatic extract of *C. caeruleum* or *M. costata* was added, in the spectrophotometer cell, to a soln of D-glucosone, 1,5-D-anhydrofructose or D-xylosone, and the mixture was scanned between 200 and 600 nm. To take into account variations of activity depending on the fungi and the substrates, optimal relative concns of enzyme and substrate were tentatively determined in each expt.

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