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OCCURRENCE AMONG MACROFUNGI OF THE BIOCONVERSION OF GLUCOSONE TO CORTALCERONE

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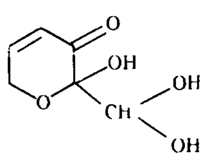
Key Word Index—*Corticium caeruleum*; *Pulcherricium caeruleum*; fungi; screening; bioconversion; glucosone; cortalcerone; enzymatic activity; chemotaxonomy.

Abstract—Bioconversion of glucosone to cortalcerone, first discovered in *Corticium caeruleum*, was found to occur in 43 macrofungi out of the 315 which were screened (13.65 %). Relations with taxonomy, ecology and biochemistry are discussed.

INTRODUCTION

We have previously reported [1] that a cultivated strain † of the lignicolous fungus *Corticium caeruleum* (Schrad. ex Fr.) Fr. (Aphyllophorales) subjected to 'activating' plasmolytic conditions produces cortalcerone, a new pyrone compound whose structure (**1**) was confirmed by X-ray crystallography [2].



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Further studies showed [3] that cortalcerone is derived from glucose through a two-step pathway involving: (i) oxidation of D-glucose to D-glucosone (D-*arabino*-2-hexosulose) by an enzymatic reaction which is presumably similar to those reported in other biological sources [3–5]; and (ii) conversion of D-glucosone to racemic cortalcerone by a mechanism involving a novel enzymatic activity. Cortalcerone production was also observed in *C. caeruleum* growing on wood, but not in cultures of several yeasts and moulds from our collection (unpublished data). These results prompted us to screen a wider range of fungi for this new bioconversion.

Cortalcerone synthesis was investigated by adding phenylhydrazine to plasmolysed fungal macerates enriched with either glucose or glucosone; cortalcerone yields a red precipitate with phenylhydrazine, but since other carbonyl molecules can give a similar reaction, more specific tests based on known properties of the compound [1] were carried out when necessary, as described in the Experimental. When only glucosone was present—either as a product of glucose oxidation or when tested as a substrate—addition of phenylhydrazine yielded almost immediately a yellow precipitate of phenylglucosazone (a reaction which is given by glucose only on heating).

RESULTS AND DISCUSSION

The 315 macrofungi which were subjected to these procedures fell into five categories which are listed in Table 1. Most of them (238, group Ia) neither produced glucosone nor cortalcerone. The 30 species of group Ib most likely oxidized glucose to glucosone‡ but did not carry out the second enzymatic step. All other fungi yielded a red precipitate with phenylhydrazine, at least in the glucosone series. In group IIa (four species), complementary tests for cortalcerone were negative, but they were positive with the 43 species of groups IIb and IIc. Thus the enzymatic conversion of glucosone to cortalcerone is not specific for *Corticium caeruleum*, and its occurrence amongst macrofungi (13.65 % of the species investigated) is not negligible.

Although this reaction seems to lack a clear chemotaxonomic significance, it should be pointed out that most of the positive species were found in Pezizales (52.4 %, with 100 % in Morels) and to a lesser extent in Aphyllophorales (22.3 %); in similar respects, the few positive Agaricales belonged mainly to the related genera *Lepista* and *Clitocybe*. No doubt this screening would be worth further studies in several taxonomic groups; electrophoretic studies of the enzymes involved should also be taken into account, since preliminary results in our laboratory showed that the two positive species *Pycnoporus cinnabarinus* and *P. sanguineus* exhibit distinct enzymatic bands.

With regards to ecology, cortalcerone production proved to be unrelated to the origin of the fungi (whenever specimens from distant areas were compared), and to their habitat; however, 40 % of the positive species were lignicolous. In other respects, no discordance was ob-

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†This strain was deposited in 1978 at the American Type Culture Collection where it is registered as *Pulcherricium caeruleum* (Schrad. ex Fr.) Parm. ATCC 36306.

‡Since this reaction did not fall within the scope of this screening, whether the yellow precipitate was really glucosazone or not was not investigated.

Table 1. Screening of 315 macrofungi for the production of cortalcerone

I. NO RED PRECIPITATE WITH PHENYLHYDRAZINE

Ia. No yellow precipitate in the glucose series

CLAVICIPITALES: *Cordyceps militaris* (2)*

SPHAERIALES: *Hypoxylon fragiforme* (1), *Diatrype* sp. (1), *Xylosphaera hypoxylon* (1), *X. polymorpha* (1), *Daldinia concentrica* (1)

HELOTIALES: *Hymenoscyphus fructigenus* (1), *Calycella citrina* (1), *Chlorosplenium aeruginosum* (1), *Leotia lubrica* (1), *Bulgaria inquinans* (2), *Neobulgaria pura* (2)

PEZIZALES: *Humaria hemisphaerica* (1), *Sarcoscypha coccinea* (3), *Tarzetta catinus* (1), *Disciotis venosa* (1), *Otidea bufonia* (2), *Verpa digitaliformis* (C1)†, *Helvella crispa* (1), *H. lacunosa* (1), *Leptopodia elastica* (2)

TUBERALES: *Tuber melanosporum* (1), *T. aestivum* (1)

DACRYMYCETALES: *Calocera viscosa* (2)

TREMELLALES: *Tremella mesenterica* (2), *Pseudohydnum gelatinosum* (1), *Guepinia helvelloides* (1), *Exidia glandulosa* (1)

APHYLLOPHORALES: *Cerocorticium confluens* (C1), *Cylindrobasidium evolvens* (C1), *Laeticorticium roseum* (C1), *Hyphoderma sambuci* (1), *Aleurodiscus aurantius* (1), *Stereum hirsutum* (2), *S. insignitum* (2), *S. rugosum* (3), *S. subtomentosum* (1), *Hymenochaete cruenta* (1), *Clavaria vermicularis* (1), *Clavariadelphus pistillaris* (1), *C. truncatus* (1), *Clavulina cristata* (1), *C. rugosa* (1), *Ramaria formosa* (1), *Sparassis crispa* (2), *Cantharellus cibarius* (2), *C. ianthinoxanthus* (1), *C. lutescens* (2), *C. tubaeformis* (1), *Craterellus cornucopioides* (1), *Pseudocraterellus sinuosus* (1), *Hydnum repandum* (2), *Steccherinum ochraceum* (1), *Thelephora terrestris* (1), *Meripilus giganteus* (2), *Laetiporus sulphureus* (1), *Polyporus badius* (1), *P. squamosus* (2), *P. varius* f. *nummularius* (1), *Piptoporus betulinus* (3), *Tyromyces caesius* (2), *Incrustoporia nivea* (1), *Hapalopilus nidulans* (1), *Trichaptum abietinus* (1), *T. fuscoviolaceus* (1), *Heteroporus biennis* (1), *Daedaleopsis confragosa* (1), *Pycnoporellus fulgens* (C2), *Ceriporia purpurea* (1), *Heterobasidion annosum* (1), *Phellinus dryadeus* (2), *Phlebia radiata* (1), *Plicatura faginea* (1), *Merulius tremellosus* (1), *Serpula lacrymans* (1), *Fistulina hepatica* (1)

BOLETALES: *Boletus edulis* (1), *B. erythropus* (2), *B. queleti* (1), *Leccinum aurantiacum* (1), *L. scabrum* (1), *Suillus bovinus* (1), *S. granulatus* (1), *Xerocomus badius* (1), *X. chrysenteron* (1), *X. versicolor* (1), *Paxillus atrotomentosus* (1), *P. involutus* (2), *P. panuoides* (1), *Hygrophoropsis aurantiaca* (1), *Omphalotus olearius* (1), *Chroogomphus rutilus* (2), *Strobilomyces floccopus* (1)

ASTEROSPORALES: *Lactarius blennius* (1), *L. controversus* (1), *L. deliciosus* (2), *L. necator* (1), *L. quietus* (1), *L. subdulcis* (1), *L. vellereus* (2), *L. volemus* (1), *Russula atropurpurea* (1), *R. cyanoxantha* (1), *R. densifolia* (1), *R. erythropoda* (1), *R. integra* (1), *R. nigricans* (1), *R. sardonia* (1), *R. turci* (1), *R. virescens* (1)

AGARICALES: *Hygrophorus agathosmus* (2), *H. eburneus* (1), *H. lucorum* (1), *H. pudorinus* (1), *Camarophyllus niveus* (1), *Hygrocybe crocea* (1), *H. punicea* (1), *Tricholoma aurantium* (1), *T. auratum* (1), *T. columbetta* (1), *T. pardinum* (1), *T. saponaceum* (3), *T. scalpturatum* (1), *T. sejunctum* (1), *T. sulphureum* (1), *T. terreum* (1), *T. ustale* (1), *T. virgatum* (1), *Tricholomopsis rutilans* (1), *Melanoleuca grammopodia* (1), *Lyophyllum connatum* (1), *L. decastes* (1), *L. ulmarium* (1), *Calocybe ionides* (1), *Laccaria amethystina* (1), *L. laccata* (2), *Clitocybe costata* (1), *C. dealbata* (1), *Lepista nuda* (2), *L. nuda* var. *glaucocana* (1), *L. sordida* (2), *Collybia butyracea* (2), *C. confluens* (1), *C. distorta* (1), *C. dryophila* (2), *C. fusipes* (1), *C. maculata* (2), *C. peronata* (1), *C. platiphylla* (1), *Oudemansiella radicata* (1), *Marasmius alliaceus* (1), *Mycena galericulata* (2), *M. inclinata* (2), *M. iodiolens* var. *tenella* (1), *M. pura* (3), *M. sanguinolenta* (1), *Pleurotus dryinus* (1), *P. ostreatus* (1), *Panus conchatus* (1), *Panellus serotinus* (1), *P. stypticus* (2), *Crepidotus variabilis* (1), *Schizophyllum commune* (3), *Hohenbuehelia geogenia* (2), *Clitopilus prunulus* (1), *Entoloma prunuloides* (1), *E. rhodopolium* (1), *E. sinuatum* (1), *Cortinarius alboviolaceus* (1), *C. bolaris* (1), *C. glaucopus* (1), *C. humicola* (1), *C. largus* (1), *C. mucosus* (1), *C. obtusus* (1), *C. praestans* (1), *C. purpurascens* (1), *C. torvus* (1), *C. trivialis* (1), *Dermocybe semisanguinea* (1), *Inocybe fastigiata* (4), *I. jurana* (1), *I. lanuginella* (1), *I. maculata* (1), *I. obscura* (1), *Hebeloma crustuliniforme* (1), *H. mesophaeum* (1), *H. sacchariolens* (2), *Gymnopilus penetrans* (1), *Rozites caperata* (1), *Agrocybe aegerita* (1), *Stropharia aeruginosa* (1), *S. rugosoannulata* (1), *S. semiglobata* (1), *Hypholoma fasciculare* (2), *H. sublateritium* (3), *Pholiota destruens* (1), *P. gummosa* (1), *P. lenta* (1), *P. squarrosa* (1), *Kuehneromyces mutabilis* (1), *Coprinus comatus* (1), *C. micaceus* (2), *Psathyrella candolleana* (1), *P. hydrophila* (1), *P. velutina* (1), *Volvariella speciosa* (1), *Pluteus curtisii* (1), *P. thomsonii* (1), *Agaricus campestris* (1), *A. placomyces* (1), *A. radicatus* (2), *A. xanthodermus* (1), *Lepiota clypeolaria* (2), *L. cristata* (1), *Macrolepiota excoriata* (1), *M. procera* (1), *M. rhacodes* (1), *Leucoagaricus pudicus* (1), *Cystoderma amianthinum* (1), *Phaeolepiota aurea* (1), *Amanita citrina* (2), *A. gemmata* (2), *A. muscaria* (2), *A. pantherina* (1), *A. phalloides* (2), *A. rubescens* (1), *A. solitaria* (1), *A. spissa* (1), *A. vaginata* (1), *A. fulva* (1)

PHALLALES: *Anthurus archeri* (1)

GASTREALES: *Cyathus striatus* (1), *Scleroderma citrinum* (1), *S. verrucosum* (1), *Lycoperdon perlatum* (1), *L. pyriforme* (1), *Geastrum sessile* (1)

Ib. A yellow precipitate in the glucose series

PEZIZALES: *Gyromitra esculenta* (1, C1)

APHYLLOPHORALES: *Peniophora incarnata* (2), *Chondrostereum purpureum* (4), *Hericium erinaceum* (1), *Phellodon niger* (2), *Grifola frondosa* (1), *Polyporus arcularius* (1), *P. brumalis* (2), *Trametes gibbosa* (1), *T. hirsuta* (2), *T. pubescens* (1), *T. trogii* (2), *T. versicolor* (4), *Datronia mollis* (3), *Lenzites betulina* (3), *Gloeophyllum abietinum* (1), *Ganoderma applanatum* (1), *G. lucidum* (2)

AGARICALES: *Tricholoma acerbum* (1), *Clitocybe phyllophila* (1), *Oudemansiella mucida* (2), *Armillariella mellea* (4), *Entoloma cetratum* (1), *E. mammosum* (3), *Inocybe abietis* (1), *I. geophylla* (2), *I. hirtella* (1), *Hebeloma sinapizans* (2), *Gymnopilus spectabilis* (2), *Pluteus murinus* (1)

II. A RED PRECIPITATE WITH PHENYLHYDRAZINE (AT LEAST IN THE GLUCOSONE SERIES)

IIa. Confirmation tests for cortalcerone negative

APHYLLOPHORALES: *Hydnellum concrescens* (2)

AGARICALES: *Lepista irina* (2), *Marasmius cohaerens* (1), *Cortinarius anomalus* (2)

IIb. Confirmation tests for cortalcerone positive, a red precipitate in both series

PEZIZALES: *Aleuria aurantia* (4), *Peziza badia* (2), *P. succosa* (1), *Sarcosphaera eximia* (1), *Morchella conica* (2), *M. costata* (2, C1), *M. elata* (2), *M. esculenta* (2, C1), *M. esculenta* var. *rotunda* (2), *M. hortensis* (C1), *Gyromitra infula* (2)

Table 1.

AURICULARIALES: *Auricularia mesenterica* (3)
APHYLLOPHORALES: *Pulcherricium caeruleum* (6, C4), *Peniophora quercina* (9, C1), *Phanerochaete sordida* (1), *Vuilleminia comedens* (1), *Stereum gausapatum* (1), *S. sanguinolentum* (1, C1), *Lopharia spadicea* (1), *Sparassis laminosa* (1), *Boletopsis subsquamosa* (1), *Bjerkandera adusta* (5), *Trichaptum biformis* (2, C2), *Cerrena unicolor* (2), *Pycnoporus cinnabarinus* (4, C2), *P. sanguineus* (1, C1), *Junghunia nitida* (2)
AGARICALES: *Clitocybe cyathiformis* (3), *C. dicolor* (1), *C. gibba* (3), *C. odora* (3), *Lepista caespitosa* (1), *L. inversa* (1), *L. luscina* (3), *L. nebularis* (3), *Mycena seynii* (4), *Pleurocybella porrigens* (1)
IIc. Confirmation tests for cortalcerone positive, a red precipitate in the glucosone series only
APHYLLOPHORALES: *Ramaria flava* (1), *Clavulinopsis helvola* (2), *C. helvola* var. *geoglossoides* (2), *C. pulchra* (2)
AGARICALES: *Marasmius oreades* (3), *Inocybe pyriodora* (1)

*(*n*): Number of specimens from distinct localities.
†(*Cn*): Number of cultivated strains tested.

served between fruit bodies from natural crops and their mycelia when cultivated as described in the Experimental.

As could be anticipated, most of the positive species produced cortalcerone from glucose as well as from glucosone, i.e. carried out both enzymatic steps (group IIb, 37 fungi). On the contrary, the six fungi of group IIc unexpectedly performed the sole second reaction, a point which would have been unnoticed if glucosone had not been tested as a substrate. It should be pointed out that four out of these six fungi are closely related (three *Clavulinopsis* and *Ramaria flava*); perhaps they contain some factor which inhibited the first activity in the experimental system, unless they simply lost it.

TLC tests with *Peziza aurantia*, *Sarcosphaera eximia*, the six positive *Morchella* and *Mycena seynii* raise another biochemical question: in addition to the spot of 2-furylglyoxal (see Experimental), another spot with a slightly lower R_f was detected by the anisaldehyde reagent, yielding a similar blue colour. We have undertaken the identification from Morels of this substance and of its precursor, the latter probably being a homologue of cortalcerone.

Although many points still remain obscure, the results of this screening draw attention to the metabolic significance of cortalcerone, and consequently to that of glucosone, which, in spite of various studies, is still far from being understood. Volc *et al.* showed not long ago [4] that in cultures of the fungus *Oudemansiella mucida* glucosone is accumulated then utilized after glucose exhaustion. Further studies should indicate whether cortalcerone or other similar compounds are intermediates in such a transformation, a result which would open a new field of investigation in glucose metabolism in fungi and perhaps in other biological systems.

EXPERIMENTAL

Fungi. Most were harvested by us in plain and mountainous areas of the south-west of France, except the tropical species *Pycnoporus sanguineus* which we brought back from the Ivory Coast. Others were obtained from the Société Mycologique du Béarn (Pau, France). Cultures of Aphyllophorales were gifts from Mme A. David (Laboratoire de Mycologie, Université Claude Bernard, Lyon, France). Cultures of Morels were purchased from Prof. J. Delmas (Station de Recherches sur les Champignons, Institut National de la Recherche Agronomique, Bordeaux, France). Nomenclature was based on refs. [6–11].

Cultures. All cultures were carried out at room temp. in daylight on yeast-extract glucose agar covered with cellophane film (as described for *Corticium caeruleum* [1]), until well-grown mycelia were obtained. All fungi were frozen prior to their treatment, which could then be delayed without perceptible loss of enzymatic activity.

Treatment of fungi. Small fragments were taken from various parts of the spore fruit, or of the mycelium in the case of cultivated fungi, and pooled. From 0.5 g up to a few g (depending on the nature of the fungus) was macerated overnight at room temp. in a minimal vol. of 0.1 % aq. solns of glucose or glucosone in two small stoppered flasks; these solns had been previously shaken with 1 % toluene, which completes the plasmolytic action of freezing–thawing and protects against bacterial contamination.

Detection of cortalcerone. Macerates were decanted or filtered, and 0.5 ml was added with an equal vol. of the phenylhydrazine reagent (phenylhydrazine, 1 ml; HOAc, 1 ml; H_2O, 8 ml). When a red ppt. was observed with the glucosone macerate, the remaining macerate was divided into two parts; one was examined for A_{max} at 230 nm, the other was made 0.1 M with HCl, heated in a steam-bath for 15 min, examined for A_{max} at 282 nm, then extracted with an equal vol. of EtOAc, which was concd and analysed by TLC for 2-furylglyoxal as previously described [1]; as the R_f is greatly influenced by the type of silica gel, it is imperative to use pure compound as a reference, or a macerate of *C. caeruleum* which has undergone the same treatment.

Glucosone. Glucosone was prepared as previously reported [3].

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