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BIOCONVERSION OF CARBOHYDRATES TO UNUSUAL PYRONE COMPOUNDS IN FUNGI: OCCURRENCE OF MICROTHECIN IN MORELS

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Key Word Index—*Morchella*; *Corticium caeruleum*; *Pulcherricium caeruleum*; fungi; morels; microthecin; cortalcerone; pyrones; 2-furylhydroxymethylketone; carbohydrates; enzymatic activity; bioconversion.

Abstract—A bioconversion similar to the one that converts glucosone to the unusual pyrone cortalcerone in *Corticium caeruleum* was shown to occur in morels, which produce the alcohol homologue, microthecin, from an unknown carbohydrate precursor.

INTRODUCTION

We recently reported [1] the screening of more than 300 macrofungi for the unusual enzymatic activity which converts D-glucosone (D-arabino-2-hexosulose) to cortalcerone (1), a pyrone previously obtained from the lignicolous fungus *Corticium caeruleum* subjected to 'activating' plasmolytic conditions [2]. To demonstrate the production of cortalcerone, we made use of its specific transformation, on hot acidic treatment, to 2-furylglyoxal (2), which is easily extracted by ethyl acetate and characterized by TLC.

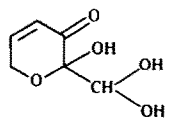
In this survey, 43 of 315 fungi examined proved to be cortalcerone-positive when activated in the presence of glucosone, i.e. yielding 2-furylglyoxal. But with some—especially morels (fresh or dried fruit bodies as well as cultivated mycelia from several *Morchella* species)—we noticed an additional component with a slightly lower R_f, which gave a similar blue colour with the anisaldehyde reagent [2]. Moreover, when these fungi were tested without adding glucosone, the latter constituent only was observed, and their macerates treated with hot acid showed an A_{max} at 275–277 nm (2-furylglyoxal: 282 nm). These data were consistent with the presence of another furan and, consequently, with the existence in the above-mentioned fungi of a cortalacerone-like substance from which it would be derived. Identification of these compounds was therefore undertaken.

RESULTS AND DISCUSSION

Isolation of the likely furan was carried out from dried fruit bodies of morels by the procedure used for 2-furylglyoxal (column chromatography followed by sublimation [2]). We thus obtained a crystalline substance identical with 2-furylhydroxymethylketone (3), i.e. the alcohol homologue of 2-furylglyoxal. Therefore, it could be anticipated that morels and all other species showing by TLC the spot of furylhydroxymethylketone contain the alcohol homologue of cortalcerone (4). We obtained this compound in pure crystalline form from cultivated mycelia of *Morchella costata*; its structure was deduced by standard spectroscopic procedures, as described in the Experimental, and was confirmed by X-ray crystallography [Carpy, A., Léger, J.-M. and Saux, M., personal communication].

This compound had previously been isolated for its antibacterial properties from cultures of *Melanospora* and *Microthecium*, and had been given the trivial name microthecin by the Japanese authors who patented it [3], apparently without correlating it with cortalcerone, with which it shares the 'dihydro-β-pyrone' ring, unusual among natural compounds [2].

Microthecin from morels and cortalcerone from *Corticium caeruleum* do not seem to be biogenetically connected by oxidoreduction, since they are not interconvertible in the presence of crude fungal enzyme extracts. Microthecin must therefore arise from a specific precursor. Although nothing is known about the mechanism of the conversion of glucosone to cortalcerone, both the latter and a possible 2,6-cyclized form of glucosone [4] have the same skeleton. On the



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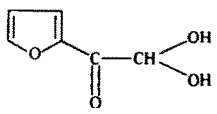

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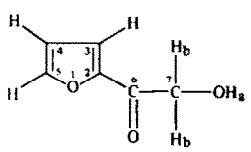

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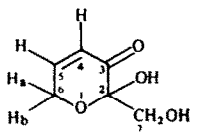

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same basis, microthecin appears to be structurally related to fructopyranose. However, testing fructose as a substrate for a crude enzymatic preparation from morels was unsuccessful. Isolation of the native substrate from these fungi was therefore undertaken and yielded a hygroscopic substance which behaves like a reducing carbohydrate but could not be crystallized. Up to now, data from mass spectrometry, ^{1}H NMR and ^{13}C NMR failed to indicate its structure, which appears to be a mixture of at least two forms.

EXPERIMENTAL

Cultivation of Morchella costata. Mycelia were grown on yeast extract–glucose agar, on a cellophane film, as those of *Corticium caeruleum* [2]; 15- to 20-day-old mycelia were then harvested and frozen.

2-*Furylhydroxymethylketone*. Dried fruit bodies (25 g) of commercial morels were macerated overnight at room temp. in 350 ml H_2O previously shaken with 3.5 ml toluene [1], then pressed, which gave 255 ml of a dark brown macerate. This liquid was made 0.1 N with HCl, refluxed for 15 min and extracted with EtOAc (2 × 200 ml). The extract was concd under red. pres., then evaporated to a brown pasty residue containing crystalline needles; this residue was dissolved in 5 ml $CHCl_3$–MeOH (93 : 7) and chromatographed on a silica gel column which bound coloured impurities (elution by the same solvent). Combined fractions showing a component at R_f 0.5 yielded, on slow evaporation, a crystalline compound which was purified by sublimation to give crystals (100 mg, losses owing to volatility), mp 78°. Found: C, 57.38, H, 4.83, 0, 37.62. Calc. for $C_6H_6O_3$: C, 57.14, H, 4.76, 0, 38.09%. UV $\lambda_{max}^{H_2O}$ nm (log ε): 223 (3.40), 275 (4.16). IR ν_{max}^{KBr} cm^{-1}: 3380 (OH), 3120 (CH of furan ring), 2930 (aliphatic CH), 1675 (conjugated C=O), 1560 (C=C), 1470, 1420, 1270, 1165, 1110, 1025, 980, 910, 880, 780, 770. ^{1}H NMR (60 MHz, $CDCl_3$): δ3.9 (1H, *m*, $W_{1/2}$ = 6 Hz, H-7a), 4.7 (2H, *s*, H-7b), 6.6 (1H, *m*, $J_{3,4}$ = 3.5 Hz, $J_{4,5}$ = 1.7 Hz, H-4), 7.3 (1H, *m*, $J_{3,5}$ = 0.7 Hz, H-3), 7.8 (1H, *m*, H-5). EIMS (probe) 70 eV, *m/z*, formula from high resoln (rel. int.): 126 $[M]^+$, $C_6H_6O_3$ (17); 95 $[M-CH_2OH]^+$, $C_5H_3O_2$ (100); 67 $[M-CH_2OH-CO]^+$, C_4H_3O (4); 39, C_3H_3 (13).

Microthecin. Fifty frozen mycelia were thawed [1] by maceration in 250 ml H_2O for 2–3 hr at room temp., then pressed and rinsed to give 300 ml of a brown liquid which was concd to 20 ml under red. pres. This dark syrupy concentrate was extracted by EtOAc (4 × 20 ml) and the combined organic liquors were evaporated to a residue which was dissolved in 5 ml MeOH. This soln was streaked on five preparative 20 × 20 cm silica gel F plates which were developed with $CHCl_3$–MeOH (7:3). Microthecin bands were detected by their strong UV absorption, pooled and eluted by the same solvent which was evaporated. The residue was dissolved in 20 ml H_2O; after filtration on a 1.2 μm membrane, lyophilization yielded 300 mg of crystalline racemic microthecin. Found: C, 49.8, H, 5.65. Calc. for $C_6H_8O_4$: C, 50.0, H, 5.55. UV λ_{max}^{dioxan} nm (log ε): 230 (3.64), 345 (1.37). IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 1690 (C=O), 1630 (C=C), 1430, 1380, 1270, 1230, 1200, 1140, 1050, 990, 950, 920, 820. ^{1}H NMR (60 MHz, Me_2CO-d_6): δ3.6 (3H, *m*, $W_{1/2}$ = 6 Hz, 2H-7 and OH-7), 4.4 (1H, *m*, $J_{6a,5}$ = 3.3 Hz, $J_{6a,4}$ = 1.3 Hz, $J_{6a,6b}$ = 19.5 Hz, H-6a), 4.6 (1H, *m*, $J_{6b,5}$ = 2 Hz, $J_{6b,4}$ = 2 Hz, H-6b), 5.3 (1H, *m*, $W_{1/2}$ = 7 Hz, OH-2), 6.0 (1H, *m*, $J_{4,5}$ = 9.8 Hz, H-4), 7.1 (1H, *m*, H-5). EIMS (probe) 70 eV, *m/z*, formula from high resolution (rel. int.): 113 $[M-CH_2OH]^+$, $C_5H_5O_3$ (6); 85, $C_4H_5O_2$ (8.5); 68, C_4H_4O (100).

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