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CORTALCERONE, A NEW ANTIBIOTIC INDUCED BY EXTERNAL AGENTS IN *CORTICIUM CAERULEUM*

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Key Word Index—*Corticium caeruleum*; fungus; antibiotic; cortalcerone; 2-hydroxy-6*H*-3-pyrone-2-carboxaldehyde hydrate; 2-furylglyoxal hydrate; induction; supraoptimal temperature; chemical intoxication.

Abstract—The antibacterial compound produced by the fungus *Corticium caeruleum* in response to external agents was identified as 2-hydroxy-6*H*-3-pyrone-2-carboxaldehyde hydrate, a new compound for which the trivial name cortalcerone is proposed.

INTRODUCTION

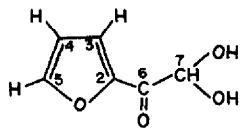
During chemical studies undertaken on the blue-pigmented, lignicolous fungus *Corticium caeruleum* (Schrad. ex Fr.) Fr. (Aphyllophorales), we have already shown that the pigment is a mixture of polymers structurally related to thelephoric acid [1], and a phenolic compound, isolated as a by-product of pigment purification, was identified as 6-*n*-pentadecyl 2,4-dihydroxybenzoic acid, a new fungal metabolite [2].

In routine antibacterial tests carried out at 24 to 25°, *C. caeruleum* did not show any detectable activity against *Staphylococcus pyogenes*. Surprisingly, antibacterial activity appeared if the mycelium had been subsequently exposed to one of the following "activating" treatments, (i) a supraoptimal temperature of 42° [3, 4], (ii) exposure to vapours of such toxic solvents as $CHCl_3$, Et_2O, Me_2CO, EtOAc [5], or (iii) contact with a H_2O–toluene mixture at room temperature. The latter procedure was retained as the most practical, and enrichment of the mixture with glucose proved more efficient, suggesting that this sugar could act as a precursor of the antibiotic compound. We also established that "activation" was effective only on mycelia which had been grown under light conditions required for the synthesis of the blue pigment by our strain of *C. caeruleum*, as we had previously reported [6].

These preliminary results prompted us to isolate and identify the antibacterial compound.

RESULTS

Strong UV absorption at 230 nm was observed in aqueous macerates of activated mycelia which showed antibacterial activity. This result is similar to that of Langvad and Goksøyr [7] in which a leakage of UV-absorbing metabolites occurred from the mycelium of *Gyrophana* (*Merulius*) *lacrymans* growing at supraoptimal temperature, which they reported as nucleic derivatives, mainly from the characteristics of the UV spectrum (λ_{max} = 260 nm). That the UV-absorbing material was also the antibiotic compound was shown by (i) white, dark-grown activated mycelia of *Corticium caeruleum* did not exhibit the 230 nm maximum, (ii) the absorption appeared in response to all the previously reported activating agents and (iii) treatment with alkali eliminated both the UV absorption and antibiotic activity. This specific UV absorption proved useful for checking chromatographic separations, and led to a purification procedure involving selective solubility and precipitations, followed by repeated Si gel column chromatography. However, these operations did not yield a pure crystalline compound, but a thick, colourless water-soluble syrup.

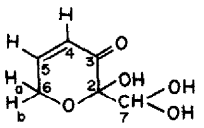


(1)

On treatment with hot acid this syrup yielded 2-furylglyoxal hydrate (1) and not sugars as we had anticipated. Analysis gave the formula $C_6H_6O_4$, and MS and IR properties were indicative of a furan ring substituted by a carbonyl group. From the PMR spectra, we concluded that there was an α-substitution by a $-CO-CH(OH)_2$ chain, the only possible structure being (1). Visual and TLC observations suggested that almost all of the syrupy material (except a few impurities) changed into (1) on treatment with acid. This fact was in favour of a close relationship between the antibiotic compound and 2-furylglyoxal hydrate, and allowed us to make various hypothesis, none of which was confirmed as long as we worked on the syrupy material.

Important information was given by the PMR spectrum of the syrupy material. It was complicated, but showed two singlets at 3.31 and 3.40 ppm (corresponding together to three protons) which could be assigned to an –OMe group bound to an asymmetric C. This fact suggested that the use of MeOH in the chromatographic

procedures could possibly result in the formation of a hemiacetal artifact from the proposed $-CH(OH)_2$ function. This would also explain the difficulty in obtaining a crystalline product from a complex mixture of stereoisomers. Dissolving the syrup in water, washing this solution with $CHCl_3$ and lyophilizing the aqueous residue were sufficient to give a yellowish solid which after purification, yielded a white crystalline compound, $C_6H_8O_5$. Its structure (2), 2-hydroxy-6*H*-3-pyrone-2-carboxaldehyde hydrate, a new compound, was deduced from MS,



(2)

IR, PMR and UV studies; the compound was obtained as a racemic form. Recrystallization from Et_2O gave crystals suitable for X-ray analysis, which was performed by another team in our Faculty; crystallographic data gave the spatial characteristics of cortalcerone molecule [8]. Studies with the pure crystalline compound confirmed its conversion to 2-furylglyoxal hydrate on acidic treatment, as the result of a loss of water, its chromatographic properties and its antibacterial properties. We propose for this new compound the trivial name "cortalcerone".

DISCUSSION

Cortalcerone appears to have an unusual structure by at least two of its chemical characteristics which, as far as we know, have not yet been reported in natural compounds: the free aldehydic hydrate group and the "dihydro-β-pyrone" ring. This type of ring exists in various *in vitro* intermediates of carbohydrates, such as unsaturated *osones* or related compounds [9–11]; it also arises from oxidation of furans [12–14] and such a reaction has recently led to the synthesis of a series of compounds, several of which showed antibacterial activity [14].

Nothing is presently known about cortalcerone biosynthesis; it could arise from glucose through a series of reactions, some of which are enzymatic and probably induced* by the "activating" agents, light being perhaps necessary to the synthesis of these inductible* enzymes; but the racemic condition of cortalcerone suggests that the ultimate step (a cyclisation?) could be a purely chemical mechanism.

EXPERIMENTAL

Cultures. The fungus was grown as previously reported [4] on an agar-solidified medium (glucose, 20 g; yeast extract, 5 g; agar, 15 g; dist H_2O, to 1 l.) distributed in Petri dishes and covered with cellophane films which helped in removing mycelia. Light conditions were those required for pigment synthesis [4].

"Activation" of mycelia. A 1‰ glucose–H_2O soln was shaken with 1% toluene. After 20 days blue mycelia were collected and floated on this soln in wide vessels (20 mycelia per l.) for 24 hr at room temp. After removing the mycelia, the aq macerate was the starting material for the preparation of cortalcerone.

2-furylglyoxal hydrate. Syrupy material (1 g) eluted from Si gel columns was refluxed for 15 min in 50 ml of 0.1 N HCl. TLC (fluorescent Si gel, $CHCl_3$–MeOH 93:7) revealed disappearance of the spot at R_f 0.3 (cortalcerone) and its substitution by a spot at R_f 0.85 giving the same blue colour on spraying with anisaldehyde reagent (anisaldehyde 0.5 ml, H_2SO_4 0.5 ml, EtOH 9 ml heating 5 min at 110°); detection also by UV at $\lambda_{254\ nm}$. Simultaneously, A_{max} at 230 nm was replaced in aq soln by a new A_{max} at 282 nm. Acidic liquor was extracted with EtOAc (3 × 50 ml) and evaporation of the extract gave a residue which was dissolved in 5 ml $CHCl_3$–MeOH (93:7). This soln was chromatographed on a Si gel column (elution by the same solvent). Combined fractions showing the spot at R_f 0.85 afforded, on slow evaporation, a crystalline compound which was purified by sublimation to give white crystals (700 mg), mp 72° (lit: 70–71°). Found: C, 51.08; H, 4.17; O, 44.86. Calc for $C_6H_6O_4$: C, 50.7; H, 4.22; O, 45.07%. UV $\lambda_{max}^{H_2O}$ nm (log ϵ): 230 (3.45), 282 (4.16). IR ν_{max}^{KBr} cm^{-1}: 3400 (O–H), 3120 (C–H of furan ring), 2980 (aliphatic C–H), 1675 (conjugated C=O), 1560 (C=C of furan ring), 1465, 1390, 1270, 1110, 1070, 1040, 970, 910, 880, 820, 770, 760. PMR [90 MHz, $(CD_3)_2CO$]: δ (TMS): 5.66 (1 H, *s*, C-7, C<u>H</u>), 5.67 [2H, *m*, $W_{1/2}$ = 10.5 Hz, C–7 (O<u>H</u>)$_2$], 6.73 (1H, *m*, J_{3-5} 0.8 Hz, J_{4-5} 1.8 Hz, C–5), 7.58 (1H, *m*, J_{3-4} 3.7 Hz, C–3), 7.93 (1H, *m*, C–4). MS (probe) 70 eV *m/e* (formula from high resoln; rel. int.): 124 (M^+–H_2O; $C_6H_4O_3$; 21), 95 [M^+–$CH(OH)_2$; $C_5H_3O_2$; 100], 67 [M^+–CO.$CH(OH)_2$; C_4H_3O; 9], 39 (C_3H_3; 43).

Cortalcerone. After removing the mycelia, the aq macerate (1 l.) was concentrated under red pres in a rotatory evaporator, at a temp not exceeding 40°, to give a syrupy product which was partially dissolved in 15 ml of MeOH; the undissolved material was separated by centrifugation and washed with MeOH. PrOH (2 vol) were added to the combined methanolic liquors and resulting ppt. eliminated by centrifugation and washed with PrOH; the combined propanolic liquors were evaporated to dryness under red pres. The residue was triturated repeatedly with 50 ml of $CHCl_3$–MeOH (93:7), the liquid filtrated to eliminate undissolved material, and concentrated to give 5 ml of a brown liquid. This soln was chromatographed on a Si gel column (elution by the same solvent) and fractions were analysed for cortalcerone (TLC on fluorescent Si gel, elution by the same solvent, detection by $A_{254\ nm}$ and by the anisaldehyde reagent, R_f = 0.3). Combined fractions were concentrated and the column chromatography repeated 2×. Evaporation of the final soln afforded a syrupy material which was dissolved in 20 ml of H_2O; this aq soln was allowed to stand for 1 hr at room temp and extracted twice with H_2O-washed $CHCl_3$ which eliminated yellow impurities. Most of the $CHCl_3$ was removed under red pres. Lyophilization of the remainder gave a yellowish crystalline solid; repeated washings with ethyl formate completed elimination of the yellow impurities and, in spite of some losses, yielded a white crystalline product, (200 mg), mp 87°. Found: C, 45.61; H, 4.97. $C_6H_8O_5$ requires: C, 45.0; H, 5.0%. UV λ_{max}^{dioxan} nm (log ϵ): 230 (3.91), 345 (1.48). IR ν_{max}^{KBr} cm^{-1}: 3400 (O–H), 1695 (C=O), 1630 (C=C), 1480, 1435, 1410, 1370, 1305, 1235, 1200, 1080, 1070, 1030, 1020, 980, 940, 810. PMR [90 MHz, $(CD_3)_2CO$]: δ (TMS): 4.37 (1H, *m*, J_{6a-6b} 19.2 Hz, J_{6a-5} 3.6 Hz, J_{6a-4} 1.8 Hz, C–6a), 4.65 (1H, *m*, J_{6b-5} 1.8 Hz, J_{6b-4} 2.4 Hz, C–6b), 4.76 (1H, *m*, $W_{1/2}$ = 10 Hz, C–2 O<u>H</u>), 5.1 [3H, *m*, $W_{1/2}$ = 5.4 Hz, C–7, C<u>H</u>, (O<u>H</u>)$_2$], 6.0 (1H, *m*, J_{4-5} 10.2 Hz, C–4), 7.2 (1H, *m*, C–5). MS (probe) 70 eV *m/e* (formula from high resoln; rel. int.): 113 ($C_5H_5O_3$; 19), 85 ($C_4H_5O_2$; 15), 68 (C_4H_4O, 100).

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* This word must not be given the precise meaning it has in molecular genetics and does not imply knowledge of the exact mechanism by which the enzymatic reactions are originated.

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