CONVERSION OF ANHYDROFUSARUBIN LACTOL INTO THE ANTIBIOTIC BOSTRYCOIDIN

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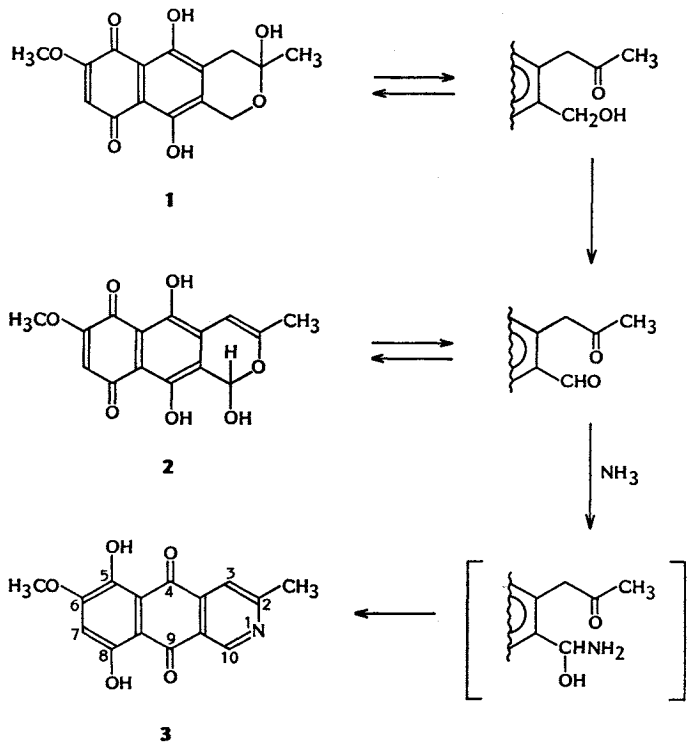
The antibiotic bostrycoidin was isolated first from the fungus *Fusarium bostrycoides*[1] and later found in a wide range of *Fusarium* species[2~4]. The structure **3** was proposed for this substance, further confirmed by total synthesis[5]. Bostrycoidin revealed interesting antibiotic properties[6], in particular being effective against *Mycobacterium tuberculosis in vitro*. According to KUROBANE *et al.*[7], bostrycoidin could originate *in vivo* from an oxidation of dihydrofusarubin in the presence of ammonia, while ARSENAULT[2] proposed a reaction between a hypothetical fusarubin aldehyde and NH_3. These hypotheses were never confirmed. Anhydrofusarubin lactol (**2**), the hemiacetal of fusarubin aldehyde, was recently identified from *Fusarium solani*[8] and from *Nectria haematococca*[9], so that it became tempting to check the last hypothesis at least *in vitro*.

When anhydrofusarubin lactol (**2**) in a benzene solution is brought to 100°C for 15 minutes in presence of an excess of concentrated ammonia (4 drops for 1 mg in a pressure tight tube) a 100% conversion into bostrycoidin is observed. The product is extracted from the reaction mixture by methylene chloride after addition of water, leading to red microcrystals (from hexane - ethyl acetate): MP 242~245°C; UV λ_{max}^{EtOH} nm (log ε) 250 (4.4), 320 (3.7), 475 (sh), 497 (3.9), 525 (sh); TLC (SiO_2) Rf 0.60 in CCl_4 - CH_2Cl_2 - MeOH, 5:1:1; high resolution MS calcd for

Fig. 1. Proposed mechanism for the formation of the antibiotic bostrycoidin (**3**) from the anhydrofusarubin lactol (**2**) (fusarubin aldehyde hemiacetal) and ammonia in fungal cultures and *in vitro*.



$C_{15}H_{11}NO_5$ 285.06371 (M), found 285.0634; ^1H NMR (400 MHz, $CDCl_3$, δ from TMS) 2.80 (3H, s, CH_3), 4.0 (3H, s, OCH_3), 6.70 (1H, s, 7-H), 7.90 (1H, s, 3-H), 9.48 (1H, s, 10-H), 13.50 (1H, s, 8-OH), 13.15 (1H, s, 5-OH); identity confirmed by direct comparison with the natural product. The anhydrofusarubin lactol (**2**) reacts slowly with ammonia even at room temperature, leading to a small amount of bostrycoidin (50% after 72 hours) so that the existence of such a reaction *in vivo* is most probable. At 60°C, the complete conversion is obtained after 1 hour. As a mechanism for this *in vitro* reaction (also occurring in cultures) we propose as a first step the formation of the aldehyde ammonia adduct as represented in the Fig. 1. Further cyclization to the oxo carbon atom followed by dehydrations would give the expected bostrycoidin (**3**).

Bostrycoidin can be obtained directly *in vitro* from fusarubin (**1**) by carrying out the oxidation with 2,3-dichloro-5,6-dicyanobenzoquinone as reported[9] and further adding ammonia to the reaction mixture. After 15 minutes warming at 100°C a 17% yield of bostrycoidin results (isolated by SiO_2 TLC in CCl_4 - CH_2Cl_2 - MeOH (5 : 1 : 1) in order to eliminate the excess reagent, then crystallization from hexane - ethyl acetate).

The ease with which bostrycoidin forms, raises the problem of its biochemical significance for the fungus. The possibility that it is an artifact formed in the culture media can not be excluded.

Fusarubinoic acid[10] formed biosynthetically from a heptaketide, appears to be a key intermediate leading through reductions to fusarubin aldehyde (**2**), and then to bostrycoidin (**3**) or to fusarubin (**1**).

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References

1) CAJORI, F. A.; T. T. OTANI & M. A. HAMILTON: The isolation of an antibiotic from *Fusarium bostrycoides*. J. Biol. Chem. 208: 107~114, 1954
2) ARSENAULT, G. P.: The structure of bostrycoidin, a β-aza-anthraquinone from *Fusarium solani* D_2 purple. Tetrahedron Lett. 1965: 4033~4037, 1965
3) STEYN, P. S.; P. L. WESSELS & W. F. O. MARASAS: Pigments from *Fusarium moniliforme* Sheldon. Structure and ^{13}C nuclear magnetic resonance assignments of an aza-anthraquinone and three naphthoquinones. Tetrahedron 35: 1551~1555, 1979
4) TATUM, J. H. & R. A. BAKER: Naphthoquinones produced by *Fusarium solani* isolated from citrus. Phytochemistry 22: 543~547, 1983
5) CAMERON, D. W.; K. R. DEUTSCHER & G. I. FEUTRILL: Synthesis of bostrycoidin and 8-*O*-methylbostrycoidin. Tetrahedron Lett. 21: 5089~5090, 1980
6) HAMILTON, M. A.; M. S. KNORR & F. A. CAJORI: Antibiotics derived from *Fusarium bostrycoides*. Antibiot. Chemother. 3: 853~856, 1953
7) KUROBANE, I.; L. C. VINING, A. G. MCINNES & N. N. GERBER: Metabolites of *Fusarium solani* related to dihydrofusarubin. J. Antibiotics 33: 1376~1379, 1980
8) TATUM, J. H.; R. A. BAKER & R. E. BERRY: Metabolites of *Fusarium solani*. Phytochemistry 28: 283~284, 1989
9) PARISOT, D.; M. DEVYS & M. BARBIER: Anhydrofusarubin lactol from *Nectria haematococca* Berk. and Br. (Wr.). Phytochemistry 28: 1989, in press
10) PARISOT, D.; M. DEVYS & M. BARBIER: Fusarubinoic acid, a new naphthoquinone from the fungus *Nectria haematococca*. Phytochemistry 27: 3002~3004, 1988