NOTIZEN

5-Deoxybostrycoidin, a New Metabolite Produced by the Fungus *Nectria haematococca* (Berk. and Br.) Wr.

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Z. Naturforsch. **44b,** 1473–1474 (1989); received July 10, 1989

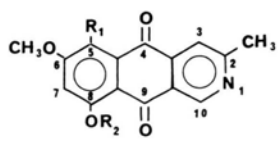
5-Deoxybostrycoidin, Fungus, *Nectria haematococca*, Pigments, Naphthoquinone

The structure **2** is established for 5-deoxybostrycoidin, a new metabolite isolated from cultures of the fungus *Nectria haematococca* (Berk. and Br.) Wr., on the basis of physico-chemical data (MS, ^{1}H and ^{13}C NMR, acetylation) and by reference to the known bostrycoidin **1.**

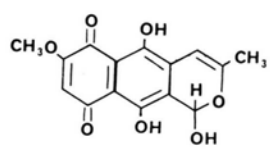
In cultures the fungus *Nectria haematococca* (Berk. and Br.) Wr., produces a series of highly coloured naphthoquinones related to fusarubin. Fourteen of these substances were previously isolated among which eight were new [1]. Recent work [2] showed that the antibiotic bostrycoidin **1** resulted from the action of ammonia on anhydrofusarubin lactol **4.** This last compound is the hemiacetal of fusarubin aldehyde and is thus immediately related to fusarubinoic acid [3], the likely precursor of fusarubin, javanicin and other naphthoquinones of the series.

For the production of the 5-deoxybostrycoidin **2,** the *169* red mutant of *Nectria haematococca* [4] was grown at 26 °C in liquid still cultures. The medium consisted of glucose 22.5 g, L-asparagine, H_2O 9.5 g, KH_2PO_4 1 g, KCl 0.75 g, $MgSO_4$, $7H_2O$ 0.5 g, $CaCl_2$, $2H_2O$ 0.15 g, $FeSO_4$, $7H_2O$ 0.003 g, $ZnSO_4$, $7H_2O$ 0.003 g, $CuSO_4$, $5H_2O$ 0.00125 g, $MnSO_4$, H_2O 0.00035 g, Na_2MoO_4, $2H_2O$ 0.00025 g, distilled water 1 liter (pH 5.5). The growth of the fungus was initiated by inoculating 25 ml of medium in Petri dishes with 100 μl of a microconidial suspension (10^6 spores per ml). After 4–5 days the medium became orange and the pH rose to 7.5–8. The culture fluid was filtered, extracted first at pH 8 with ethyl acetate till the extracts became pale yellow and then reextracted after acidification to pH 3 with acetic acid. A red and a yellow major pigments were found in both extracts after SiO_2 TLC in CH_2Cl_2–MeOH 99:1 (red product, bostrycoidin **1,** Rf 0.53, yellow substance, **2,** 5-deoxybostrycoidin, Rf 0.70, while standard fusarubin had Rf 0.32). Preparative TLC's in the same conditions gave 45 and 6 mg of these two substances per liter of culture filtrate of the *169* mutant of *N. haematococca*, respectively.

The red compound was identified as bostrycoidin **1** on the basis of MS, ^{1}H NMR, Rf, mp., by comparing with a standard of this product. The substance **2** was crystallized from CH_2Cl_2-hexane giving yellow needles, mp. 195–196 °C, IR (KBr) 1680, 1635, 1590 cm^{-1}, UV (MeOH) 207, 237, 270, 322 (sh.), 414 nm, characteristic values for the 1,4-naphthoquinone moiety in **2.** The molecular formula $C_{15}H_{11}NO_4$ was established by MS (*m/z* 269, M^+) and by elemental analysis: calc. C 66.91, H 4.12, N 5.20%; found C 66.89, H 4.32, N 4.93%. The ^{1}H NMR ($CDCl_3$, 220 MHz, δ ppm from TMS) allowed the attribution of all protons: 2.78, s, 3H, (CH_3); 3.94, s, 3H, (OCH_3); 6.74, d, J = 2.5 Hz, 1H, H–C-7; 7.32, d, J = 2.5 Hz, 1H, H–C-5; 7.86,



1 R_1 = OH R_2 = H
2 R_1 = R_2 = H
3 R_1 = H R_2 = CH_3CO



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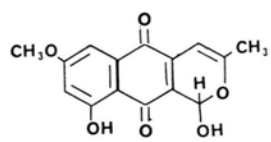

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Figure. **1:** bostrycoidin; **2:** 5-deoxybostrycoidin; **3:** 5-deoxybostrycoidin acetate; **4:** anhydrofusarubin lactol; **5:** 5-deoxyanhydrofusarubin lactol (still hypothetical).

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Verlag der Zeitschrift für Naturforschung, D-7400 Tübingen
0932–0776/89/1100–1473/$ 01.00/0

s, 1H, H−C-3; 9.40, s, 1H, H−C-10; 12.76, s, 1H, OH at C-8. The relative position of the aromatic protons in the cycle A of **2** was further established by irradiation at 7.32 ppm (H−C-5), resulting in the decoupling (and enhancement) of the signal at 6.74 (H−C-7, meta-coupling). The position of all signals and in particular for H−C-3 and 10 was further corroborated by direct comparison with the ^{1}H NMR spectrum of bostrycoidin **1.** ^{13}C NMR ($CDCl_3$): 165.6 (C-2), 118.5 (C-3), 134.6 (C-3a), 186 (C-4), 138.7 (C-4a), 107.4 (C-5), 165.9 (C-6), 108.1 (C-7), 166.54 (C-8), 110.5 (C-8a), 182 (C-9), 124 (C-9a), 149.1 (C-10), 25.3 (CH_3), 56.1 (OCH_3). Acetylation of **2** (Ac_2O/Py 20 h, 20 °C, SiO_2 TLC in CH_2Cl_2−MeOH 99:1, Rf 0.55) gave a yellow monoacetate **3,** mp. 193−195 °C, MS *m/z* 311, M^+, 269, M-42 (100%). The ^{1}H NMR spectrum of **3** was similar to that of **2** except for the aromatic protons at C-5 and C-7 which were deshielded at 7.72 and 6.97 ppm respectively and for the OH group at C-8 which was replaced by the CH_3CO signal at 2.52 (s, 3H).

Previous work was done with the *169* red mutant of *N. haematococca* grown in a medium containing ten times less asparagine (0.95 g/l) than in the present study. In such conditions the pigments found were the usual dihydroxynaphthoquinones (fusarubin, hydroxynorjavanicin, bostrycoidin and others) [1−5] and the dihydrodihydroxynaphthoquinones (dihydrofusarubins, dihydrofusarubin-O-ethyl ether) [5−6]. No monohydroxynaphthoquinones have been isolated from cultures of red mutants. Monohydroxynaphthoquinones were obtained only when an additional chromosomal mutation was introduced into such red mutants, giving yellow double mutants [7]. The monohydroxynaphthoquinones: deoxyfusarubin, deoxyanhydrofusarubin and deoxyjavanicin replaced fusarubin, anhydrofusarubin and javanicin in cultures of these yellow mutants [8]. Till now, the simultaneous production of both monohydroxy and dihydroxynaphthoquinones was reported only for a *Fusarium sp.* strain grown in a malt extract-glucose-peptone medium [9]. In the present work, the use of a large amount of asparagine in the culture medium inhibits the biosynthesis of fusarubin and of the other nitrogen-free dihydroxynaphthoquinones but allows the formation of bostrycoidin together with a small quantity of its monohydroxy counterpart namely, 5-deoxybostrycoidin. The accumulation of bostrycoidin may be explained by the fact that asparagine furnishes ammonia so that the fusarubin aldehyde (hemiacetal **4**) is immediately transformed into the heterocyclic derivative according to the previously proposed scheme [2]. The origin of deoxybostrycoidin is presently unclear. A likely hypothesis is that it arises from a hypothetical deoxyfusarubin aldehyde **5** (hemiacetal form).

The melting points were determined on a Kofler apparatus under the microscope and were corrected, the MS were obtained from an AEI MS 9 spectrometer and the NMR spectra from Bruker 220 and 400 MHz apparatus, δ ppm from TMS as internal standard.

Thanks are due to Drs. B. C. Das and C. Girard for mass spectrometric determinations, to Mrs. C. Fontaine and C. Pasquier for the ^{1}H and ^{13}C NMR spectra, to Mrs. C. Muller for elemental analysis, and to Mrs. M. Maugin (Orsay) and L. Quaino (Gif sur Yvette) for their expert technical assistance.

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