Phytochemistry, Vol. 28, No. 11, pp. 3240–3241, 1989.
Printed in Great Britain.

0031-9422/89 $3.00+0.00

ANHYDROFUSARUBIN LACTOL FROM *NECTRIA HAEMATOCOCCA*

DENISE PARISOT, MICHEL DEVYS* and MICHEL BARBIER*

Laboratoire de Cryptogamie, Bâtiment 400, Faculté des Sciences, 91405 Orsay Cedex, France; *CNRS, Institut de Chimie des Substances Naturelles, Avenue de la Terrasse, 91198 Gif sur Yvette Cedex, France

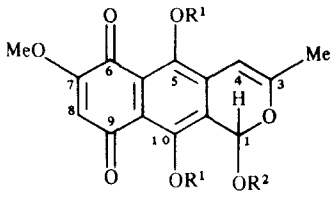
(*Received in revised form* 14 *March* 1989)

Key Word Index—*Nectria haematococca*; fungus; naphthoquinones; anhydrofusarubin lactol.

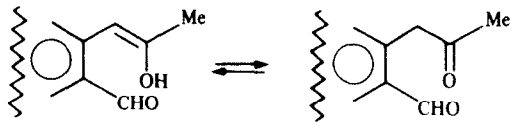
Abstract—Anhydrofusarubin lactol has been isolated from the fungus *Nectria haematococca* and identified on the basis of its spectral properties, the formation of derivatives and a partial synthesis from fusarubin. Its biological origin is discussed.

The recent identification of 1,5,10-trihydroxy-7-methoxy-3-methyl-1H-naphtho-[2–3*c*]-pyran-6,9-dione (**1**) from *Fusarium solani* [1] prompts us to report our own results concerning this substance which we had isolated from *Nectria haematococca* Berk. and Br. (Wr.) and for which we propose the name of anhydrofusarubin lactol. This new violet pigment was identified by us on the basis of spectral determinations [MS, ^{1}H and ^{13}C NMR on **1** and its diacetate (**3**) and methyl ketal (**4**)].

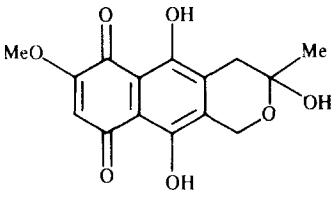
The oxidation of fusarubin (**5**) by dichlorodicyanobenzoquinone (DDQ) gives a small amount (*ca* 10%) of anhydrofusarubin lactol (**1**), together with some of the



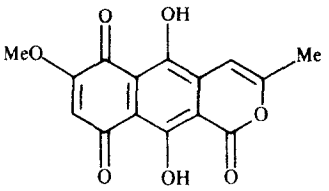
1 $R^1 = R^2 = H$
3 $R^1 = Ac$, $R^2 = H$
4 $R^1 = H$, $R^2 = Me$



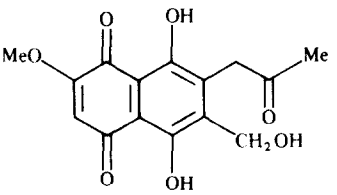
2a **2b**

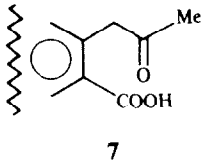


5



6





7

DDQ → **2a**, **2b**, **1**

methyl ketal **4** (formed from the action of methanol during the purification procedures) and a series of unidentified products. Anhydrofusarubin does not furnish compound **1** when submitted to a parallel reaction under the same conditions. It seems quite probable that the lack of reactivity of DDQ towards the phenolic OH groups is due to their chelation with the quinone functions.

The biological origin of anhydrofusarubin lactol (**1**) through enzymatic reduction of the oxo group in anhydrofusarubin lactone (**6**) [2] cannot be excluded. However, oxidation of the primary benzylic alcohol resulting from the opening of the hemiketal pyran ring in fusarubin (**5**) represents another possibility. The attack of such benzylic alcohols by mild oxidizing agents, in particular by DDQ, to give aldehydes is known to occur [3, 4]. Indeed, it is very likely the mechanism which is involved in our partial synthesis of **1**. A most remarkable fact is the very early formation of anhydrofusarubin lactol (**1**) in cultures of *N. haematococca*. This occurs before any significant biosynthesis of fusarubin so that the formation of **1** from fusarubin seems unlikely. This leads to the proposal that **1** is formed by reduction of the carboxylic acid group of fusarubinoic acid (**7**), a compound which is itself most probably formed by cyclization of the heptaketide [5]. A further reduction of the aldehyde would lead to the alcohol, the open form of the hemiketal pyran. The above proposals, which can be summarized as follows: acetate → heptaketide → **7** → **2** (a, b) → **1**, await verification.

EXPERIMENTAL

Anhydrofusarubin lactol (**1**) appears in young cultures of *N. haematococca* a few hours before any detectable amounts of fusarubin [6]. TLC of extracts from 3- to 4-day-cultures of 6 different strains were examined. All strains synthesized anhydrofusarubin lactol in variable amount. The **958** red mutant which produces **1** in higher quantities than fusarubin was chosen as the source of the pigment. The culture of the organism and the extraction of the pigment were performed as previously described [5]. Anhydrofusarubin lactol (**1**) was isolated from the hexane–EtOAc (9:1) extract of the culture filtrate and purified by repeated precipitation by hexane from CH_2Cl_2. By using this direct procedure and excluding CC and TLC, 23 mg of **1** and 19 mg of fusarubin were obtained per l of culture medium after 4 days of growth of the organism.

Anhydrofusarubin lactol (**1**). Amorphous dark violet powder; UV λ_{max}^{MeOH} nm (log ε): 204 (3.51), 226 (3.58), 260 (3.59), 275sh, (3.52), 340 (2.93), 510sh, (3.33), 534 (3.37), 570sh, (3.20); R_f 0.30 in CCl_4–CH_2Cl_2–MeOH (5:1:1), 0.40 in CH_2Cl_2–MeOH (49:1); MS *m/z* (rel. int.): 304 $[M]^+$ (31) (calc. for $C_{15}H_{12}O_7$ 304.0579, found 304.0582), 289 (11) $[M-15]^+$, 287 (10) $[M-17]^+$, 286 (34) $[M-18]^+$ 273 (8) $[M-MeO]^+$, 262 (40) $[M-42]^+$ (ketene from the oxo form of the open pyran ring), 261 (100) $[M-MeCO, id.]^{+\cdot}$ ^1H NMR, pyridine-d_6: δ2.20 (3H, *s*, Me–3), 3.96 (3H, *s*, MeO-7), 6.36 (1H, *s*, H-8), 6.57 (1H, *s*, H-4), 6.83 (1H, *s*, H-1), 12.88 (1H, *s*, OH-10), 12.93 (1H, *s*, OH-5); $(CD_3)_2SO$: 2.08 (3H, *s*, Me-3), 3.90 (3H, *s*, MeO-7), 6.12 (1H, *s*, H-8), 6.50 (1H, *s*, H-4), 6.42 (1H, *d*, H-1), 7.72 (1H, *d*, OH-1), 12.60 and 13.30 (phenolic OH groups). The signals of the three OH groups disappear upon addition of D_2O.

Diacetate **3**. Acetylation of **1** in pyridine–Ac_2O, 20 hr, 20°, afforded (from TLC) a mixture of products in which the diacetate **3** is the predominant compound; amorphous, R_f 0.70 in CH_2Cl_2–MeOH (49:1); ^1H NMR, $CDCl_3$: δ2.13 (3H, *s*, Me-3), 3.96 (3H, *s*, MeO-7) 2.56 (6H, *s*, MeCO), 6.07 (1H, *s*, H-8), 6.57 (1H, *s*, H-4), 6.87 (1H, *s*, H-1), 6.60 (1H, *brs*, OH-1).

Methyl ketal **4**. Compound **1** was dissolved in absolute MeOH (10 mg/1 ml) and 3 drops of MeOH–H_2SO_4 (4 drops H_2SO_4/1 ml MeOH) were added. After 1 hr at 20°, compound **4** was extracted with EtOAc–H_2O. The EtOAc phase was washed with H_2O, dried over Na_2SO_4 and left to give red purple microcrystals, mp 186–190° (dec.). TLC shows (R_f 0.75 in CCl_4–CH_2Cl_2–MeOH 5:1:1) that the transformation of **1** into **4** was quantitative. Demethylation to give **1** was obtained (100%) by gentle warming of a MeOH–H_2O soln of **4** in the presence of a drop of H_2SO_4. **4**: MS *m/z* (rel. int.): 318 (35) $[M]^+$, 286 (100) $[M-32\ (MeOH)]^+$; ^1H NMR, IR, UV-Vis spectra as reported [1]. ^{13}C NMR ($CDCl_3$): 6 CH atoms and 10 non-CH atoms are present: 20.63 (Me), 55.87 (Me, C-1), 56.75 (OMe, C-7), 94.50 (CH, C-4), 94.79 (CH, C-1), 161.31 (C-3), 121.36 (C-4a), 158.46 (C-OH, C-5), 107.75 (C-5a), 175.02 (C-6), 159.87 (C-7), 110.06 (C-8), 180.29 (C-9), 111.48 (C-9a), 161 (C-OH, C-10), 132.67 (C-10a). Assignements made by comparison with reported values in the series [7, 8].

Partial synthesis of anhydrofusarubin lactol (**1**). A suspension of 10 mg of fusarubin in 4 ml dry benzene was introduced into a pressure-tight tube and 40 mg of dichlorodicyano-benzoquinone (DDQ) in 1 ml benzene was added. The mixture was kept for 45 min in an oven at 100°. The resultant homogenous reaction product was cooled and submitted to prep. TLC (CCl_4–CH_2Cl_2–MeOH, 5:1:1); **1** was recovered as a violet band emerging from the reagent zone (R_f 0.30) while the starting fusarubin had disappeared. The product was eluted from the silica gel with EtOAc and $CHCl_3$ and further submitted to a second prep. TLC (CH_2Cl_2–MeOH, 49:1) (R_f 0.38, 1.1 mg, *ca* 10%). The corresponding methyl ketal **4** was also prepared from **1** as mentioned. The formation of the methyl ketal was observed as a secondary reaction occurring during the extraction of the silica gel. It results from an interaction between **1** and MeOH in the solvent system.

Acknowledgements—Thanks are due to Drs B. C. Das and C. Girard for mass spectrometry, to Mrs C. Pasquier and C. Fontaine for ^1H and ^{13}C NMR spectra carried out at Gif sur Yvette and to Mrs L. Quaino (Gif) and M. Maugin (Orsay) for expert technical assistance.

REFERENCES

1. Tatum, J. H., Baker, R. A. and Berry R. E. (1989) *Phytochemistry* **28**, 283.
2. Parisot, D., Devys, M., Férézou, J. P. and Barbier M. (1983) *Phytochemistry* **22**, 1301.
3. Becker, H. D. and Adler A. (1961) *Chem. Scand.* **15**, 218.
4. Lee, H. and Harvey R. G. (1983) *J. Org. Chem.* **48**, 749.
5. Parisot, D., Devys, M. and Barbier M. (1988) *Phytochemistry* **27**, 3002.
6. Parisot D. (1988) *Exp. Mycol.* **12**, 35.
7. Parisot, D., Devys, M. and Barbier M. (1985) *Phytochemistry* **24**, 1977.
8. Kurobane, I., Zaita, N. and Fukuda, A. (1986) *J. Antibiot.* **39**, 205.