Tetrahedron Letters,Vol.25,No.33,pp 3575-3578,1984 0040-4039/84 $3.00 + .00
Printed in Great Britain

NECATORONE, AN ALKALOIDAL PIGMENT FROM THE GILLED TOADSTOOL LACTARIUS NECATOR (AGARICALES)[1]

Burkhard Fugmann, Bert Steffan and Wolfgang Steglich*

Institut für Organische Chemie und Biochemie der Universität Bonn,
Gerhard-Domagk-Straße 1, D-5300 Bonn, Germany

Summary: Necatorone, an alkaloid possessing an unusual 5,10-dihydroxy-dibenzo[de,h][1,6]naphthyridin-6-one structure has been isolated from fruit-bodies of the toadstool Lactarius necator.

The dark olive-brown fruit-bodies of Lactarius necator (Bull. em Pers. ex Fr.) Karst. [= L. turpis (Weinm.) Fr.] change to a deep purple when exposed to ammonia. This was ascribed to the presence of a pigment related to polyporic acid as early as 1896[2]. In continuation of our studies of fungal pigments we elucidated the structure of necatorone, one of the pigments responsible for this colour reaction.

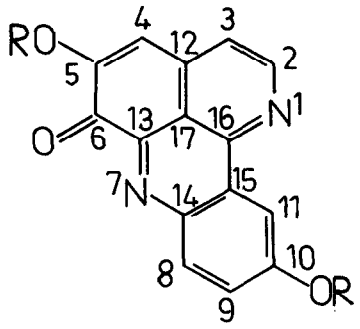
The isolation of the pigments presented considerable difficulties due to their instability and the presence of large quantities of mannitol. We found that the best procedure for the isolation of necatorone was as follows. The lyophilized and defatted peeled skins from the caps and stalks of L. necator were extracted with liquid ammonia to yield after evaporation a greyish-violet powder, which was dissolved in water. After removal of lipoids by extraction with ethyl acetate, the aqueous phase was brought to pH 5-6 with boric acid. Exhaustive extraction with ethyl acetate yielded a golden-yellow solution[3], which was further purified by prep. TLC (silicagel 60, Merck, $CHCl_3$/MeOH 2:1). The olive coloured zone was extracted and chromatographed on a column filled with Sephadex LH-20 (acetone/MeOH 4:1; argon as protecting gas, 0-4°C). From the main yellow-coloured fraction, necatorone was obtained in the form of red needles on recrystallization from methanol. By this procedure 16 mg of pure

pigment were obtained from 9 kg of fresh toadstools[4,5]. The compound dissolves in DMSO with a grass-green colour, showing strong green-yellow fluorescence. On careful addition of aqueous ammonia a change to a beautiful blue colour is observed, which turns to purple on addition of excess ammonia.

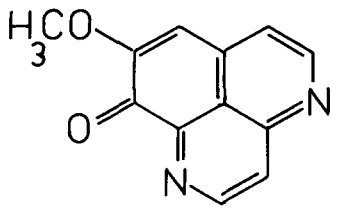
Necatorone showed the following properties: no m.p. up to 360°C; R_F 0.62 (silicagel 60; $CHCl_3$/MeOH 2:1)[6a], 0.73 (iPrOH/HCO_2H/H_2O 20:1:5)[6b].- UV/Vis (MeOH): λ_{max} (log ε) = 431 (4.13), 310 (sh, 3.85), 293 (3.88), 265 (sh, 4.13), 233 (4.60), 212 nm (sh, 4.38); (+NH_3): 557, 420, 335 (sh), 307, 253 (sh), 234, 213 nm (sh). (+ excess NH_3): 610 (sh), 520, 400, 327, 270 (sh), 248 nm. - FT-IR (KBr)[7]: 3530 (s), 3440 (s, br.), 3280 (s), 2955 (s), 2928 (ss), 2857 (s), 1685 (m, sh), 1646 (s), 1610 (ss), 1520 (m), 1460 (m), 1436 (s), 1392 (s), 1366 (m), 1297 (w), 1265 (w), 1250 (m), 1210 (s), 1185 (s), 1140 (m), 1120 (m), 1065 (m), 997 (w), 975 (w), 880 (w), 870 (w), 840 (w), 820 (w), 727 (w), 699 (w), 645 (w), 566 (w), 516 cm^{-1} (w). - ^1H NMR ([D_6]DMSO, 400 MHz): δ = 6.96 ppm (s, 1H), 7.50 (dd, J = 9.0+2.7 Hz, 1H), 7.66 (d, J = 4.5 Hz, 1H), 8.23 (d, J = 9.0 Hz, 1H), 8.33 (d, J = 2.7 Hz, 1H), 9.03 (d, J = 4.5 Hz, 1H). - MS (70 eV, 240°C): m/z 266 (M^++2, 15.0%), 265 (M^++1, 11.2), 264.0539 (M^+, calc. for $C_{15}H_8N_2O_3$: 264.0535, 26.3), 237 (23.8), 236 ($C_{14}H_8N_2O_2$, 100), 220 (6.2), 208 (29.4), 181 (8.8), 180 (11.2), 179 (14.4).

For the structural elucidation of the pigment, the coupled ^{13}C NMR spectrum (table 1) was most informative. Its systematic analysis, assisted by extensive ^1H,^{13}C -decouplings, leads unequivocally to structure 1 for necatorone, which can be systematically named as 5,10-dihydroxy-dibenzo[de,h][1,6]naphthyridin-6-one.

In accord with this formula, 1 can be methylated with excess diazomethane in methanol/water to yield the dimethyl ether 2 as the main product. 2 forms a brown-yellow solid, which exhibits an intensive green fluorescence in organic solvents and gives a positive Dragendorff test. - ^1H NMR (CD_2Cl_2, 400 MHz): δ = 3.97 ppm (s, 3H), 4.01 (s, 3H), 6.34 (s, 1H), 7.22 (d, J = 5.6 Hz, 1H), 7.23 (dd, J = 9+2.5 Hz, 1H), 7.38 (d, J = 9 Hz, 1H), 8.18 (d, J = 2.5 Hz, 1H), 8.49 (d, J = 5.6 Hz, 1H).



1 R = H
2 R = CH_3



3

Table 1. ^{1}H-coupled ^{13}C NMR spectrum of necatorone ([D_6]DMSO, 100.2 MHz)

C	δ(^{13}C) [ppm]	multiplicity, J [Hz]	H	δ(^{1}H) [ppm]
C-2	154.16	Dd, 180.4+3.2	H-2	9.03
C-3	120.93	Ddd, 170+8+3.6	H-3	7.66
C-4	111.08	Dd, 162.8+5.5	H-4	6.96
C-5	154.19	d, 4.4		
C-6	178.81	d, 7.0		
C-8	133.75	D, 163.6	H-8	8.23
C-9	122.53	Dd, 162+5.0	H-9	7.50
C-10	160.05	ddd, 9.2+2+2		
C-11	105.79	Dd, 163+4.8	H-11	8.33
C-12	137.65	d, 6.2		
C-13	143.41	s		
C-14	141.54	dd, 8.4+6.0		
C-15	128.40	d, 5.6		
C-16	145.34	ddd, 14+12+3		
C-17	113.86	ddd, 7+8+2		

Necatorone represents a new type of alkaloid which has structural similarities to demethyloxyaaptamine 3, an antimicrobially active compound from a sea sponge[8]. Recently, a Finnish group described a highly mutagenic red

compound from L. necator, for which a 7-hydroxycoumaro[5,6-c]cinnoline structure was proposed[9]. This compound, 'necatorin', has the same molecular composition as 1 and shows the same fragments in the mass spectrum. The possibility that 'necatorin' is closely related or identical with 1 can not be excluded. In contrast to 'necatorin', necatorone showed no evidence for mutagenicity in the Ames test[10].

Acknowledgements: We are grateful to the Deutsche Forschungsgemeinschaft for financial support of this work and funding of a Bruker WM 400 NMR spectrometer. B. F. thanks the Fonds der Chemischen Industrie for a doctoral fellowship.

NOTES AND REFERENCES

1. Pigments of Higher Fungi, 46; 47. H. Anke, I. Casser, R. Herrmann and W. Steglich, Z. Naturforsch., in print.

2. V. Harlay, Bull. trim. Soc. Mycol. (France) **1896**, 156.

3. The insoluble brown residue gives a blue-violet colour reaction with aqueous NH_3. However, we were unable to draw any structural conclusions from the badly resolved IR and NMR spectra of this material.

4. The same compound was obtained by extraction of the fruit-bodies with methanol/acetone, although in much lower yield.

5. Collected in October 1982 and 1983 near Idstein, Taunus, Germany.

6. a) olive-green spot, + NH_3 → violet, + conc. H_2SO_4 → red; b) yellow spot, + NH_3 → violet, + H_2SO_4 → orange.

7. We thank Prof. W. Kelker and Mr. Grußbach, Hoechst AG, for these measurements.

8. H. Nakamura, H. Wu, R. Abe, J. Kobayashi, Y. Ohizumi and Y. Hirata, 26th Symposium on the Chemistry of Natural Products, Symposium Papers, p. 118, Kyoto 1983; cf. Chem. Abstr. **100**, 83029y (1984).

9. T. Suortti, A. von Wright and A. Koskinen, Phytochemistry 22, 2873 (1983); T. Suortti and A. von Wright, J. Chromatogr. 255, 529 (1983).

10. We thank Prof. T. Anke, Universität Kaiserslautern, for this result. The Ames test was carried out without the addition of rat liver microsomes.

(Received in Germany 7 May 1984)