mixture extracted with petrol (1 l. × 3) and then with $CHCl_3$ (1 l. × 3). The $CHCl_3$ soluble fraction (173 g) was chromatographed on a silica gel column. Elution with solvents of increasing polarity yielded **1a** [1], amarolide, acetylamarolide [2] and 100 mg of **1b**.

2-Dihydroailanthone (**1b**). White prisms, mp 262–263° (MeOH), IR ν_{max} cm^{-1}: 3500–3300, 1715, 1620 and 980. UV λ_{max} nm: 205. ^1H NMR and ^{13}C NMR (pyridine-d_5): see Tables 1 and 2. MS m/z 378 $[M]^+$, 266 and 250. (Found C, 63.18; H, 6.90. $C_{20}H_{26}O_7$ requires C, 63.48; H, 6.93 %.)

Acetylation of **1b**. A soln of 2-dihydroailanthone (**1b**) (50 mg), pyridine (2 ml) and Ac_2O (0.5 ml) was kept at room temp. for 48 hr. Usual work up gave **2** (55 mg) which was crystallized from EtOH (Mp 234–236°). ^1H NMR ($CDCl_3$): see Table 1. MS m/z 486 $[M - 18]^+$.

Sodium borohydride reduction of **1a**. A soln of 0.1 g of **1a**, 0.18 g of Na_2CO_3 and 0.03 g of $NaBH_4$ in 50 ml of H_2O was kept for 72 hr at 0°. The reaction mixture was filtered on Amberlite IR 120 (in H^+ form) column; the resin was then eluted with MeOH and the eluate evaporated. Chromatography of the residue on silica gel and elution with $CHCl_3$–MeOH (95:5) yielded 30 mg of semi-solid **1c**. ^1H NMR and ^{13}C NMR (pyridine-d_5): see Tables 1 and 2. UV λ_{max} nm: 205.

Acknowledgements—We thank Professor A. M. Giuliani (C.N.R., Rome) for recording the ^1H NMR spectrum of **1c**. This work was supported partly by the Ministero della Pubblica Istruzione of Italy.

REFERENCES

1. Casinovi, C. G., Ceccherelli, P., Grandolini, G. and Bellavita, V. (1964) *Tetrahedron Letters* 3991.
2. Polonsky, J. and Fourrey, J. L. (1964) *Tetrahedron Letters* 3983.
3. Casinovi, C. G., Bellavita, V., Grandolini, G. and Ceccherelli, P. (1965) *Tetrahedron Letters* 2273.
4. Stocklin, W., Stefanovic, M., Geissman, T. A. and Casinovi, C. G. (1970) *Tetrahedron Letters* 1970.
5. De Carneri, I. and Casinovi, C. G. (1968) *Parassitologia (Rome)* 215.
6. Casinovi, C. G., Fardella, G., Grandolini, G. and Burinato, C. (1981) *Il Farmaco Ed. Sci.* 116.
7. Pettit, C. R. and Cragg, G. M. (1978) *Biosynthetic Products for Cancer Chemotherapy* Vol. 2. Plenum Press, New York.
8. Polonsky, J. and Bourguignon-Zylber, N. (1965) *Bull. Soc. Chim. Fr.* 2793.
9. Gottlieb, H. E., Hagaman, E. W. and Wenkert, E. (1975) *J. Org. Chem.* **40**, 2499.
10. Noara, H., Furuno, T., Ishibashi, M., Tsuyuki, T., Takahashi, T., Itai, A., Iitaka, Y. and Polonsky, J. (1982) *Chem. Letters* 661.

Phytochemistry, Vol. 22, No. 12, pp. 2873–2874, 1983.
Printed in Great Britain.
0031-9422/83 $3.00 + 0.00

NECATORIN, A HIGHLY MUTAGENIC COMPOUND FROM *LACTARIUS NECATOR*

TAPANI SUORTTI, ATTE VON WRIGHT and ARI KOSKINEN*

Technical Research Centre of Finland, Food Research Laboratory, Biologinkuja 1, SF-02150 Espoo 15, Finland; *Technical University of Helsinki, Department of Chemistry, SF-02150 Espoo 15, Finland

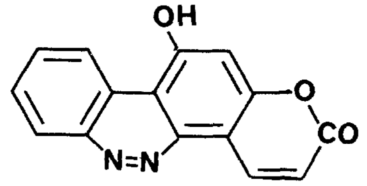
(*Revised received* 25 *January* 1983)

Key Word Index—*Lactarius necator*; Russulaceae; fungi; mutagenic compound; coumarin-cinnoline.

Abstract—The mutagen, necatorin, has been obtained from *Lactarius necator* and identified as 5-hydroxycoumaro (7,8-*c*)cinnoline.

Several species of mushrooms in the genus *Lactarius* have been found to contain mutagenic compounds in the Ames/*Salmonella* assay [1]. By far the highest mutagenic activity was found in *Lactarius necator* (Fr.) Karst. We have previously reported [2] the isolation of a mutagenic compound in crystalline form by HPLC of an extract of *Lactarius necator*. In the present communication we report its tentative identification.

Interpretation of the mass spectrum reveals the compound to be a coumarin on the basis of two sequential losses of carbonyl ($[M - CO]^+$ is the base peak) [3]. The coumarin structure is further confirmed by the photosensitizing effect of the compound; this effect is similar to that of several furanocoumarins [4]. This structure is further confirmed by two doublets in the ^1H NMR spectrum at δ 6.55 and δ 7.55 ($J = 9$ Hz) assigned to protons three and four in a coumarin system. The benzo(c)cinnoline structure is revealed by the loss of nitrogen after two losses of carbonyl in the mass spectrum. The benzo(c)cinnoline structure is in accordance with the presence of a four proton system in the ^1H NMR spectrum. The third loss of carbonyl is indicative of the hydroxyl group, which is confirmed by proton exchange in the ^1H NMR spectrum in CF_3COOD. The presence of a



Necatorin

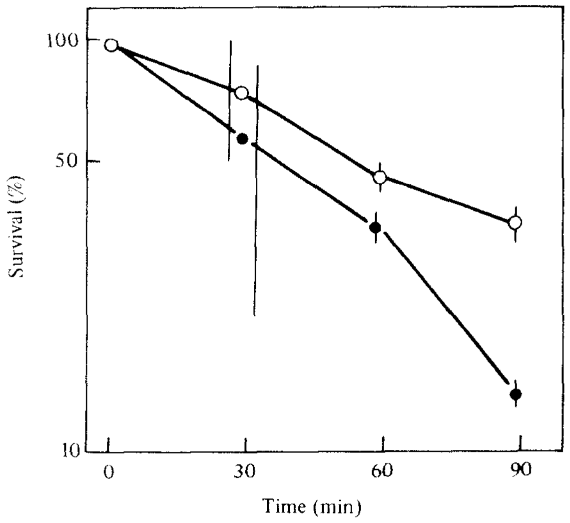


Fig. 1. Photosensitizing effect of necatorin on the bacterium *Salmonella typhimurium* TA 100. (O) Survival of bacteria suspended in saline containing necatorin (2 μg/ml). (●) Survival of bacteria exposed to necatorin and irradiated with 360 nm light. The bars indicate the s. d. of three experiments.

broad singlet at δ 7.30 in ^{1}H NMR excludes the possibility of a proton in the β-position in relation to the diazo group. The absence of a proximity effect [5], that would lead to loss of a diazo group instead of nitrogen, excludes also the possibility of a hydroxyl group β to the diazo group. To further elucidate the structure, the lactone ring was opened by raising the pH to 10 and closed again by lowering it to 4.5. In HPLC the compound remained as a single peak. Since the isolation procedure also employs lactone ring opening and closing, the isolated compound would be a mixture of possible isomers. Thus the absence of proximity effects also excludes 5-hydroxycoumarins. Boiling of the compound in 0.5 N NaOH for 3 hr had no effect on it which suggests that it has neither *para-* nor *ortho*-hydroxyl groups. All these data suggest the structure of 7-hydroxycoumaro(5,6-c)cinnoline to the compound called necatorin. The structure is further supported by the abundant occurrence of various 7-mono-oxygenated and the absence of 5- or 6-mono-oxygenated coumarins in nature.

EXPERIMENTAL

Crystalline necatorin (4.8 mg) was isolated from 30 kg *Lactarius necator* (Fr.) Karst. mushrooms as described previously [2], mp 220–225° (d); FDMS emitter current 20 mA, m/z 264; CIMS (ammonia, probe) m/z 265 $[M+H]^+$; EIMS (probe) 70 eV, m/z (rel. int.): 264.053 (27); $[M]^+$ ($C_{15}H_8N_2O_3$ requires 264.053), 236.058 (100) $[M-CO]^+$ ($C_{14}H_8N_2O_2$ requires 236.058), 208.063 (21) $[M-CO-CO]^+$ ($C_{13}H_8N_2O$ requires 208.063), 207.052 (10) $[M-CO-CO-H]^+$ ($C_{13}H_7N_2O$ requires 207.056), 180.059 (8) $[M-CO-CO-N_2]^+$ ($C_{13}H_8O$ requires 180.057), 179.062 (11) $[M-CO-CO-CO-H]^+$ ($C_{12}H_7N_2$ requires 179.061), 152 (4) $[M-CO-CO-N_2-CO]$, 151 (4) $[M-CO-CO-CO-H-N_2]^+$ or $[M-CO-CO-N_2-CO-H]^+$.

HPLC for separation of isomeric forms. M-6000A pump. M-710B injector, RCM-column chamber with C_{18} column (100 × 8 mm with 5 μm particles) and dual M-440 UV detector monitoring at 254 and 405 nm (all from Waters Assoc.). Eluent was 20 mM H_3PO_4–KOH buffer (pH 3.5)–MeOH (9:11) at a flow rate of 1 ml/min. UV λ_{max}^{MeOH} nm: 425, 310 s, 265 s, 235; $\lambda_{max}^{H_2O(NaOH\ pH\ 10)}$ nm: 520, 325, 245; ^{1}H NMR (60 MHz, CF_3COOD): δ6.55 (1H, *d*, *J* = 9 Hz), 7.30 (1H, *br s*), 7.55 (1H, *d*, *J* = 9 Hz), 8.31 (1H, *m*), 6.5–7.8 (3H, m); IR ν_{max}^{KBr} cm^{-1}: 3350 (OH), 3375 (NH tautomeric with OH proton) 1650 (C=O), 1610 (Ar-H), 1230 (C–O–C–O)

Photosensization studies. The bacterium used was *Salmonella typhimurium* TA 100 [6]. Exponentially growing cells were suspended in physiological saline containing necatorin (2 μg/ml). Half of the suspension was preserved as unirradiated control and the other half was irradiated in an open Petri dish with 360 nm light from TLC reading apparatus. Aliquots were withdrawn from both the irradiated and control suspensions at regular intervals and the viable bacterial counts were determined.

The mushrooms were identified by Mr. M. J. Pellinen in this institute.

Acknowledgements—We are indebted to Dr. E. Larsen of Risø Forsogsanleg, Denmark, for FDMS, and to Dr. J. Taskinen and Mr. P. Ottoila of Orion Oy, Finland, for CIMS and high resolution EIMS.

REFERENCES

1. Knuutinen, J. and v. Wright, A. (1982) *Mutation Res.* **103**, 115.
2. Suortti, T. and v. Wright, A. (1983) *J. Chromatogr.* **255**, 529.
3. Porter, Q. N. and Baldas, J. (1971) *Mass Spectrometry of Heterocyclic Compounds* p. 147. Wiley–Interscience. New York.
4. Musajo, L. and Rodighiero, R. (1972) in *Photobiology* (Giese, A. C., ed.) p. 115, Academic Press, New York.
5. Bowie, J. H., Lewis, G. E. and Reiss, J. A. (1968) *Aust. J. Chem.* **21**, 1233.
6. McCann, J. Spingarn, N. E., Kobori, J. and Ames, B. N. (1975) *Proc. Nat. Acad. Sci. U.S.A.* **72**, 979.