Tetrahedron Letters No. 13, pp. 1195 - 1196, 1972. Pergamon Press. Printed in Great Britain.

ON A NEW UNUSUAL METABOLITE FROM IRPEX PACHYODON (PERS) QUEL.

G.M. Nano and M. Bellando
Istituto di Chimica farmaceutica e tossicologica and Istituto botanico dell'Università
C.so Raffaello 31, Torino (Italy)

(Received in UK 7 February 1972; accepted for publication 17 February 1972)

From the benzene extracts of culture filtrates and from cultivated mycelium (1) of Irpex pachyodon (Pers) Quel., a wood basidiomycete with recently discovered antibiotic properties (2), we have isolated an antibiotically inactive, white crystalline compound with mp 89-91° and a pK_a of 9,1. The present communication gives evidence of structure 1 (methyl acethydroxamyl acetate) for this metabolite.

$CH_3-CO-NHO-CH_2-COOCH_3$ 1

Elemental and mass spectral analyses established the molecular formula as $C_5H_9NO_4$ (3). The I.R. spectrum (KBr) exhibits bands at 3170,1658,1510 cm^{-1} attributed to a secondary amide group and bands at 1750, 1240 cm^{-1} assigned to an ester group. The N.M.R. spectrum of the metabolite shows four signals (2.00 ppm s 3H, 3.82 ppm s 3H, 4.55 ppm s 2H, 9.73 ppm br 1H) of protons not coupled to each other. The peaks at 2.00 and 3.82 ppm indicate two methyl groups, of the secondary amide and of the methyl ester respectively. The peak at 4.55 ppm can be ascribed to a methylene group lying between -COOR and -OR' groups, and the broad band at 9.73 ppm can be assigned to a -NH-group. The Mass spectrum also supports these findings.

All reported data suggest formula 1, but some uncertainty remains with respect to the hydroxamic oxygen atom, since the spectral data do not give unequivocal information on the presence of the -N-O-C- group, and another structure 2 cannot be excluded.

$CH_3-CO-N(OH)-CH_2-COOCH_3$ 2

The metabolite was synthesized by reaction of methyl bromoacetate with potassium acethydroxamate (4). It is known that this kind of reaction gives preferentially O-alkyl-hydroxamate (5), and this is supported by the fact that both natural and synthetic products do not react with ferric chloride as compound 2 would do. Moreover,

when the metabolite was warmed with 6 N HCl and the volatile products of hydrolysis were removed by steam during the reaction, methanol and acetic acid were found in the distillate. At the same time we obtained in good yields from the reaction liquor a product with mp 151-53° (dec.), identified as carboxymethoxylamine hemichloride by comparison with an authentic sample (6). Evidence was thereby obtained for the presence of the -N-O-C group and the above described chemical and spectral data confirm the structure 1.

Studies on the biogenesis and the role of this rather unusual metabolite are at present in progress. The analogies and the differences with the well known metabolism of hadacidine (7) are of interest.

REFERENCES AND FOOTNOTES

(1) The mycelium was cultivated on malt liquid medium at 37°.

(2) M. Bellando, M.A. Bianco and J. Ceruti Scurti, Allionia 16, 1 (1970).

(3) Elemental analysis: found C 40.76, H 6.10, N 9.39 - calcd C 40.81, H 6.17, N 9.52. Mass spectrum (direct inlet 20°, 70 eV): M/e^+ 147,116,105,73,59,43 (base peak),31.

(4) Fairly good yields (60-65%) of the crude product mp 87-89° can be obtained by refluxing a methanolic solution of 1.0 mM of potassium acethydroxamate with 1.5 mM of methyl bromoacetate for 15 min. and by extracting the reaction mixture with hot benzene after the methanol has been removed.

(5) See e.g.: Houben Weil; Methoden der organischen Chemie (IV edition), Vol.8 p. 690 and Vol.10/I p. 1183 or F. Mathis, Bull. Soc. Chim. France, (1953), D 9 .

(6) The compound has been synthesized as in: Organic Syntheses, Collective Vol. III, Wiley, 1955, p. 172.

(7) R.L. Stevens and F.T. Emery, Biochem., 5, 74 (1966) and F.T. Emery, Antib., 22, 17 (1967).