Journal of Labelled Compounds and Radiopharmaceuticals—Vol. XXVII, No. 1

LABELLING OF OCHRATOXINS WITH ^{3}H OR ^{125}I

D.Schmiedová, K.Vereš, B.Černý

Institute of Nuclear Biology and Radiochemistry,
Czechoslovak Academy of Sciences, Prague

J. Ruprich, Institute of Hygiene and Epidemiology, Prague

J. Němeček, Institute of Microbiology, Czechoslovak Academy of Sciences, Prague

SUMMARY

Catalytic hydrogenation of ochratoxin A by carrier-free tritium was used to prepare ^{3}H-ochratoxin B with a high specific activity. Iodination of ochratoxin B by carrier-free $Na^{125}I$ using the chloramine method yielded ^{125}I-ochratoxin with a high specific activity. Another ^{125}I-derivative of ochratoxin A was prepared by iodination of an ochratoxin A - L--tyrosine-methylester conjugate. All three radioactive preparations were found to be useful for radioimmunoassay.

Key words: Ochratoxin A and B, labelling, tritiation, radioiodination, tyrosine-methylester conjugate

INTRODUCTION

Ochratoxins are secondary metabolites of various fungi, e.g. Aspergillus ochraceus Wilhelm. They are mycotoxins which cause a variety of endemic diseases, both of animals and of humans, after intake of contaminated food or fodder.

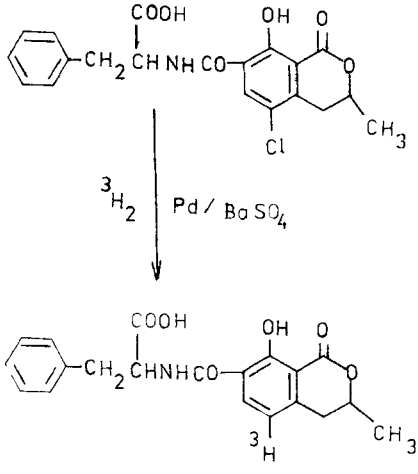
0362–4803/89/010051–07$05.00

Received November 3, 1988
Revised June 10, 1988

This study is concerned with the preparation of ochratoxin derivatives labelled with ^{3}H or ^{125}I with a high specific activity which makes them useful as radioligands in radioimmunoassay.

The preparation of nonspecifically labelled ^{3}H-ochratoxin A by catalytic exchange was described by Chang and Chu (1) who obtained a preparation with a specific activity of 96 - 111 GBq/mmol. ^{3}H-Ochratoxin with a specific activity of 3.7 TBq/mmol labelled in the phenylalanine moiety of the molecule has recently been described by Hult (2) who used condensation of ochratoxin α with ^{3}H-phenylalanine ethyl ester.

Our method of preparation of ^{3}H-ochratoxin includes a catalytic hydrogenolysis of a chlorine atom in the molecule of ochratoxin A 1 , which yields [5-^{3}H]ochratoxin B 2a .

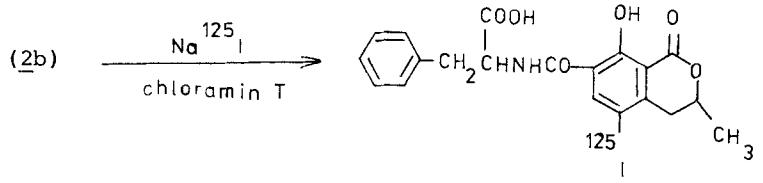


1

2a

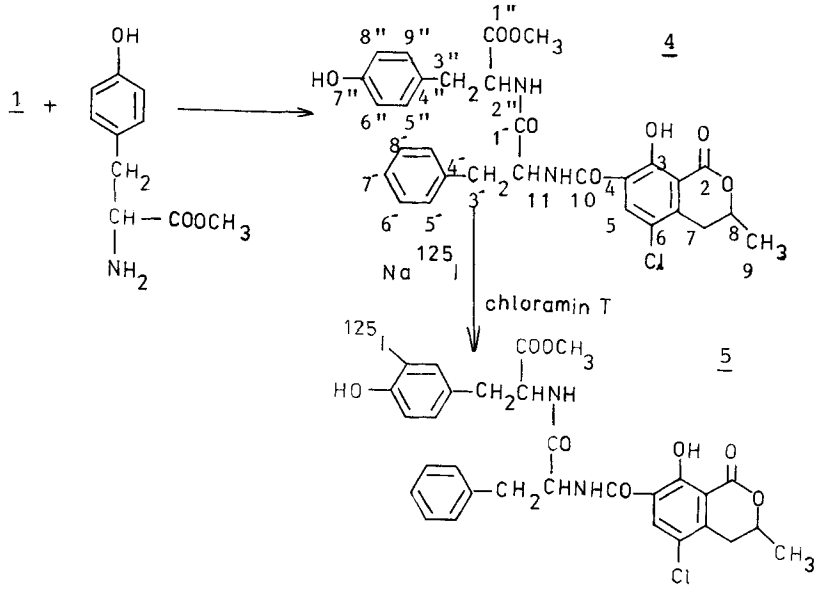
On using carrier-free tritium we obtained the product with a yield of 70.6 % and a specific activity of 1.12 TBq/mmol.

Nonradioactive ochratoxin B 2b , when iodinated according to Hunter and Greenwood, yields carrier-free [5-^{125}I]ochratoxin B 3 with a radiochemical yield of 34 %.



3

The third radioactive product 5 was obtained by iodination of the conjugate 4 of ochratoxin A with L-tyrosine methylester.



All three radioactive compounds were found to be useful as radioligand for radioimmunoassay.

EXPERIMENTAL

The work was performed with ochratoxin A (SERVA) and Na^{125}I (Isotope Production and Reactor Centre, Poland).

TLC was carried out on DC Alufolien Kieselgel $60_{F\ 254}$ (Merck), detection was done at 254 and 366 nm under a UV-lamp.

Tritium activity was measured on Beckman LS 7800. ^{125}I activity was measured on Beckman Gamma 8500 counter. The radiochemical purity of the /5-^{3}H/ochratoxin B was stated using TLC linear analyser Berthold LB 2832.

Mass spectrometry was carried out on Jeol MS 100 or MAT 44S instruments, NMR spectrometry on Varian VXR-400, and UV spectrometry was carried out on Specord UV/VIS.

/5-^{3}H/Ochratoxin B 2a

To the solution of 1.1 mg ochratoxin A in 200 µl methanol was added 9 mg sodium acetate and 10.6 mg 10 % $Pd/BaSO_4$ and the mixture was hydrogenated with carrier-free tritium for 30 min. Repeated lyophilization of the solvent (1. methanol, 2. t-butanol : H_2O = 9 : 1) removed labile radioactivity. The residue was dissolved in methanol, the catalyst was removed by centrifugation and the mixture was purified by preparative TLC (chloroform : ethyl acetate : : formic acid = 60 : 40 : 1). The zone corresponding to /^{3}H/ochratoxin B was eluted with methanol and the mass of the product was measured by UV spectrometry. The yield of /^{3}H/ochratoxin B was 0.76 mg (70.6 %) with a specific activity of 1.12 TBq/mmol. The purity of the product was verified by analytical TLC in the systems chloroform : ethyl acetate : : formic acid = 60 : 40 : 1 and benzene : methanol : acetic acid = 90 : 5 : 5. The product corresponded to the non-radioactive standard of ochratoxin B and contained no radioactive impurities.

/5-^{125}I/Ochratoxin B 3

To 10 µl solution of ochratoxin B in dioxane (1 mg/5 ml) was added 10 µl 0.05 M phosphate buffer (pH 7.5), 10 µl

carrier-free $Na^{125}I$ (37 MBq) and 10 µl solution of chloramine T in 0.05 M phosphate buffer (5 mg/ml). The mixture was agitated for 3 min and the reaction was then stopped by the addition of 10 µl solution of sodium hydrosulphite in 0.05 M phosphate buffer (5 mg/ml). The product was extracted with 2 x 100 µl ethyl acetate and isolated by preparative TLC in the system chloroform : ethyl acetate : formic acid (60 : 40 : 1). Product zone was detected by autoradiography and eluted with methanol. The radiochemical yield of the iodination was 42 %. The radiochemical purity of the product was verified by analytical TLC (benzene : methanol : acetic acid = 90 : 5 : 5) and by following autoradiography. No detectable radioactive impurities were found.

Conjugate of ochratoxin A with L-tyrosine methylester 4

To the solution of 20 mg ochratoxin A in 1 ml dioxane was added 18 µl tributylamine and 7 µl isobutylchloroformate. The mixture was stirred for 1 h in an ice-cold bath and supplemented dropwise with a solution of 19.5 mg L-tyrosine methyl ester in 1 ml dioxane, and after another 4 h under stirring another 5 mg L-tyrosine methyl ester. The solvent was removed by evaporation on the next day, the residue was dissolved in 10 ml ethyl acetate and extracted with 1 N HCl, saturated solution of $NaHCO_3$ and water. After drying and removing ethyl acetate by evaporation we obtained 20 mg residue which was purified by preparative TLC in the system chloroform : ethyl acetate : formic acid (60 : 40 : 1). Two zones were separated (12 mg; 4.6 mg) and their samples were subjected to hydrolysis by HCL (4 h, 110 °C). Both isolated products were found to contain tyrosine and phenylalanine (TLC in the system BuOH : CH_3COOH : H_2O = = 4 : 1 : 1, detection with 0.2 % ninhydrin in acetone).

Both products 4a, 4b were purified by preparative TLC in the system benzene : methanol : acetic acid = 20 : 1 : 1.

Mass spectrometric analysis of the compound 4a demonstrated the presence of ions m/z 581 and 537 which correspond to protonated molecules and ions (MH minus CO_2). The most frequent among negative ions are ions with m/z 255 (100 %) and 254 (50 %), both of them probably containing one chlorine atom, and ions with m/z 580 (40 %, $M^{-\cdot}$), 579 (35 % $(M-H)^-$) and 544 (40 %, $(M-HCl)^{-\cdot}$). The chemical ionization spectra show that the substance has a molecular weight of 580 which is in keeping with the proposed structure of the condensation product.

^1H NMR (400 MHz, $CDCl_3$, TMS, 25 °C) of the compound 4a: 1.581 d (3H, H-9, J(8,9) = 6.4); 2.831 dd (1H, H-7a, J(7a, 7b) = 17.4, J(7a, 8) = 11.7); 2.870 dd (1H, H-3"a, J(2",3"a) = 5.8, J(3"a, 3"b) = 14.0); 3.025 dd (1H, H-3"b, J(2", 3"b) = 5.2, J(3"a, 3"b) = 14.0); 3.146 dd (1H, H-3´a, J(2´, 3´a) = 7.2, J(3´a, 3´b) = 13.9); 3.210 dd (1H, H-3´b, J(2´, 3´b) = 6.9, J(3´a, 3´b) = 13.9); 3.280 dd (1H, H-7b, J(7a, 7b) = 17.4, J(7b,8) = 3.4); 3.683s (3H, $COOCH_3$); 4.790 ddq (1H, H-8, J(7a, 8) = 11.7, J(7b, 8) = 3.4, J(8, 9) = = 6.4); 4.807 m (1H, H-2", J(2", 3"a) = 5.8, J(2",3"b) = 5.2, J(2",11´) = 8.0); 4.912 m (1H, H-2´, J(2´, 3´a) = 7.2, J(2´,3´b) = = 6.9, J(2´, 11) = 7.3); 6.534 and 6.729 AA´BB´(4H, H-5", 6",8",9", J(AB) + J(AB´) = 8.5); 7.200-7.330 m (5H, H-5´,6´, 7´, 8´, 9´); 8.353 d (1H, H-11, J(2´, 11) = 7.3); 8.362s (1H, H-5).

^1H NMR (400 MHz, $CDCl_3$, TMS, 25 °C) of the compound 4b: 1.604d (3H, H-9, J(8,9) = 6.3); 2.867dd (1H, H-7a, J(7a,7b) = = 17.4, J(7a,8) = 11.6); 2.951dd (1H, H-3"a, J(3"a,3"b) = = 14.1, J(2",3"a) = 6.2); 2.989dd (1H, H-3"b, J(3"a,3"b) =

= 14.1, J(2",3"b) = 5.6); 3.137dd (1H,H-3´a, J(2´,3´a) = 6.4, J(3´a, 3´b) = 14.0); 3.239dd (1H,H-3´b, J(2´,3´b) = 6.8, J(3´a, 3´b) = 14.0); 3.288dd (1H, H-7b, J(7a,7b) = 17.4, J(7b,8) = 3.4); 3.692s (3H, $COOCH_3$); 4.764m (1H, H-8, J(7a,8) = 11.6, J(7b,8) = 3.4, J(8,9) = 6.3); 4.809m (1H, H-2", J(2",3"a) = 6.2, J(2",3"b) = 5.6, J(2",11") = 7.9); 4.931m (1H,H-2´, J(2´,3´a) = 6.4, J(2´,3´b) = 6.8, J(2´,11´) = 7.8); 6.467d (1H, H-11´, J(2´, 11´) = 7.9); 6.550 and 6.720 AA´BB´(4H, H-5", 6",8",9", J(AB) + + J(AB´) = 8.2); 7.190-7.320m (5H, H-5´, 6´, 7´, 8´, 9´); 8.353d (1H,H-11, J(2´,11) = 7.8); 8.372s (1H,H-5).

Observed ^1H NMR spectra of both compounds agree with the structure of the conjugate of ochratoxin A and tyrosine methyl ester. Small differences in chemical shifts and coupling constants of tyrosine aliphatic protons indicate an inversion of configuration at C-2" during the condensation.

The conjugate 4a was radioiodinated in the same way as ochratoxin B. Reaction time was 90 s, product was isolated by TLC in the same system, radiochemical yield was 34 %.

Acknowledgment

Authors wish to thank Dr.V.Hanuš, CSc., from the J.Heyrovský Institute of Physical Chemistry and Electrochemistry for mass spectrometric analysis, and Mrs.H.Trnková for skilled technical assistance.

References

1. Chang F.C., Chu F.S.:
J.Labelled Compounds Radiopharm. 12:231-38 (1976).

2. Hult K.:
J.Labelled Compounds Radiopharm. 23:801-5 (1986).