



HAL
open science

Contribution of uniformly ^{13}C -enriched sterigmatocystin to the study of its pulmonary metabolism

Odile Cabaret, Olivier Puel, Françoise Botterel, Michel Péan, Stéphane
Bretagne, Marcel Delaforge

► To cite this version:

Odile Cabaret, Olivier Puel, Françoise Botterel, Michel Péan, Stéphane Bretagne, et al.. Contribution of uniformly ^{13}C -enriched sterigmatocystin to the study of its pulmonary metabolism. *Rapid Communications in Mass Spectrometry*, 2011, 25 (19), pp.2704-2710. 10.1002/rcm.5068 . hal-02643815

HAL Id: hal-02643815

<https://hal.inrae.fr/hal-02643815v1>

Submitted on 28 May 2020

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Rapid Commun. Mass Spectrom. 2011, 25, 2704–2710
(wileyonlinelibrary.com) DOI: 10.1002/rcm.5068

Contribution of uniformly ^{13}C -enriched sterigmatocystin to the study of its pulmonary metabolism[†]

Odile Cabaret^{1,2}, Olivier Puel³, Françoise Botterel^{1,2}, Michel Pean^{4,5,6}, Stéphane Bretagne^{1,2} and Marcel Delaforge^{7*}

¹UMR BIPAR, U-PEC, AFSSA, ENVA, Faculté de Médecine, Créteil Cedex F-94010, France

²AP-HP, Groupe hospitalier Henri Mondor-Albert Chenevier, Laboratoire de Parasitologie-Mycologie, Créteil Cedex F-94010, France

³ToxAlim INRA, Equipe d'Immunologie-Toxicologie, Toulouse F-31027, France

⁴CEA, DSV, IBEB, Groupe de Recherches Appliquées en Phytotechnologie, Saint-Paul-lez-Durance, F-13108, France

⁵CNRS, UMR Biologie Végétale & Microbiologie Environnementale, Saint-Paul-lez-Durance, F-13108, France

⁶Aix-Marseille Université, Saint-Paul-lez-Durance, F-13108, France

⁷CEA Saclay, iBiTec-S, SB2SM and URA CNRS 2096, Gif sur Yvette Cedex F-91191, France

Mycotoxins are secondary metabolites of filamentous fungi which can cause a wide range of systemic effects. Human health effects of inhaled mycotoxins remain poorly documented, despite the large amounts present, associated with airborne particles. Among these mycotoxins, sterigmatocystin is one of the most prevalent. Because its chemical structure is close to that of the aflatoxins, we studied its metabolism and its cellular consequences when in contact with the airway epithelium, using the mass spectral signature from the 10% ^{13}C uniformly enriched sterigmatocystin. The metabolism was studied *in vitro*, using recombinant cytochrome P450s enzymes, and in porcine tracheal epithelial cell (PTEC) primary cultures at an air-liquid interface. The metabolites were analyzed by high-performance liquid chromatography coupled with tandem mass spectrometry detection. Expressed enzymes and PTECs were exposed to uniformly ^{13}C -enriched sterigmatocystin to confirm the relationship between sterigmatocystin and its metabolites because this isotopic cluster shape is conserved for all metabolites and their product ions. Incubation of sterigmatocystin with recombinant cytochrome P450 1A1 led to the formation of three metabolites identified as monohydroxysterigmatocystin, dihydroxysterigmatocystin and one glutathione adduct, the latter after the formation of a transient intermediate. In the PTEC cultures, sterigmatocystin metabolism resulted in a glucuro-conjugate. Two other products were detected, a sulfo-conjugate and a glucuro-conjugate of hydroxysterigmatocystin upon cytochrome P450 1A1 induction. This is the first study to report sterigmatocystin metabolism in airway epithelium, and it suggests that, contrary to the aflatoxins, sterigmatocystin is mainly detoxified into its conjugates and is unable to produce significant amounts of reactive metabolites in respiratory cells, at least in pigs. Copyright © 2011 John Wiley & Sons, Ltd.

Mycotoxins are secondary metabolites of filamentous fungi which can cause a wide range of acute and chronic systemic effects. As xenobiotics, these compounds can be metabolized by some of the host enzymes. These enzymes, primarily hepatic cytochromes P450 (CYP), ensure the oxidation of many hydrophobic exogenous compounds, increasing their polarity and thus assuring their biliary or urinary elimination. This transformation can involve a reduction of the parental compound toxic effects (reduction of ergotism by oxidation of the ergot alkaloids, e.g.^[1]) or, in other cases, can involve a toxic activation, as in the case of aflatoxins.^[2] These modified compounds are then conjugated to polar compounds in conjugation reactions. Glucuronic acid moieties

are transferred to substrate molecules that contain oxygen, nitrogen, sulfur or carboxyl groups. Sulfonic acid moieties are transferred from a donor molecule to an acceptor alcohol or amine and the conjugation of reduced glutathione to electrophilic centers, on a wide variety of substrates.

Most publications on mycotoxins deal with hazards due to toxic effects after their gastrointestinal uptake.^[3] There are fewer data available concerning toxicity and metabolism after inhalation, despite the detection of mycotoxins in bioaerosols and dust at workplaces and in homes^[4,5] and their association with inhaled spores.^[6,7]

Among mycotoxins, sterigmatocystin (STG) is one of the most prevalent in the environment. STG has been detected in carpet dust from damp dwellings^[8] and in dwellings after water flooding.^[9,10] *Aspergillus versicolor* is the most efficient producer of STG, which is a biochemical precursor in the biosynthesis of aflatoxin.^[11] After ingestion, STG is carcinogenic in animal models^[12–14] and it is classified as possibly carcinogenic in humans.^[15] STG is activated by CYP to a liver carcinogen.^[16] To date, STG metabolites have never been studied in airway epithelium.

* Correspondence to: M. Delaforge, CEA Saclay, iBiTec-S, SB2SM and URA CNRS 2096, Gif sur Yvette Cedex F-91191, France.

E-mail: marcel.delaforge@cea.fr

[†] Presented at the 6th Congress of the French Society of Stable Isotopes (Société Française des Isotopes Stables, SFIS) held 26–29 October 2010 in Toulouse, France.

The aim of this study was to elucidate the metabolism of STG after contact with airway epithelium and to assess any possible cellular consequences. For this purpose, we used uniformly ¹³C-enriched STG to confirm the metabolic relationship to STG. Preliminary studies consisted of identifying STG metabolites in recombinant CYP enzymes, and thus the metabolism upon exposure of porcine tracheal epithelial cell (PTEC) primary cultures to STG.

EXPERIMENTAL

Chemicals

Ham's F-12 nutrient medium, Dulbecco's modified Eagle's medium, penicillin/streptomycin, amphotericin B, fetal calf serum, 0.05% trypsin-EDTA (1X) and phosphate-buffered saline were purchased from Invitrogen Life-Technologies (Cergy-Pontoise, France). Gentamicin, collagen IV, commercial ¹²C STG (molecular weight (MW) = 324.3) with purity of 98%, acetonitrile, reduced nicotinamide adenine dinucleotide phosphate (NAPDH), glutathione (GSH), β-naphthoflavone, and ammonium acetate were obtained from Sigma (Saint Quentin Fallavier, France). Ultrosor G was purchased from BioSeptra SA (Cergy-Saint-Christophe, France); dimethyl sulfoxide (DMSO) from ProLabo (VWR International, Fontenay sous Bois, France).

Production of uniformly ¹³C-enriched STG

Production of uniformly ¹³C-enriched STG was performed using autoclaved 10%-¹³C wheat grains (*Triticum aestivum* cv. Ardente) as the exclusive nutrient source for the fungal culture, as previously described.^[17] The grains were moistened to a water activity value of 0.98 before sterilization. Around 30 g of this plant material was inoculated with a suspension of 2.10⁵ *Emericella nidulans* conidia. STG was extracted from 14-day-old cultures with chloroform. The raw extract was fractionated by automated flash chromatography (Teledyne Isco, Lincoln, NE, USA). The fractionation was performed on a normal-phase RediSep 12 g column (Teledyne Isco) at a flow rate of 25 mL/min with linear gradient. The solvent system was 10% acetic acid in toluene as solvent A and 10% acetic acid in ethyl acetate as solvent B. Initially solvent B was present at 0% and increased to 45.5% within 10 min. The fraction volume was set to 5 mL. The presence of STG in fractions was checked by high-performance liquid chromatography (HPLC) with diode-array detection (Kontron Instruments, Neufahrn, Germany) with a 250 × 4.6 mm Modul-cart Strategy 5 μm C18-2 column (Interchim, Montluçon, France). Gradient chromatography (run of 46 min) was performed with 0.2% acetic acid in water (eluent A)/acetonitrile (eluent B) as the mobile phase at a flow rate of 1.5 mL/min. The compounds were eluted by starting from 31% solvent B for 30 min followed by a linear gradient up to 90% within 5 min. After an isocratic elution for 5 min, the gradient was reduced to its initial value within 5 min, and remained at this value for the final 5 min. The ¹³C STG was purified from positive fractions by semi-quantitative HPLC. The HPLC unit was fitted with a 250 × 7.8 mm Modul-cart Strategy 5 μm C18-2 column (Interchim) and a diode-array detector. The gradient program was the same as above, except that the flow rate was increased to 4.5 mL/min. After production of uniformly ¹³C-enriched STG

the presence of an isotopic cluster close to that expected from theoretical evaluation^[17] was checked by mass spectrometry (MS).

Metabolite studies by human cytochrome P450 enzymes

Bactosome[®]-expressed human cytochromes P450 (CYP) were obtained from Cypex (Tebu-bio, Le Perray en Yvelines, France). We tested the main human CYP differing by their active site and thus by their substrate specificity.^[18] Based on the structural homology of STG with aflatoxins, we considered the CYP isoforms 1A, 2A and 3A, reported to metabolize aflatoxin B₁.^[19–22] The metabolism of both commercial ¹²C STG and ¹³C STG was studied as previously described.^[23]

STG metabolism in porcine tracheal epithelial cells (PTECs)

Preparation of porcine primary cultures was performed with trachea obtained from the Centre de Recherche Chirurgicale of the Henri-Mondor Hospital (Créteil, France) as previously described.^[24] After proliferation during the first week, the PTECs reached the differentiated state during a second week of culture with the presence of an apical side at an air interface and a basal side at a liquid contact. PTECs were exposed when cell differentiation was stable (after 14 days of culture) for 48 h to 1 mL of 1 μM U10% ¹³C STG through the basal side of the culture. After this exposure the medium was collected for extraction. To examine the effect of amounts of CYP1A1, the cells were pre-treated for 24 h with 25 μM β-naphthoflavone, a well-known CYP1A inducer, which led to an increase of CYP1A expression, or with 0.2% DMSO as control. The basal medium was then removed and the STG incubated for an additional 48 h. Controls were obtained after 48 h incubation with ¹³C STG in coated wells without cells and with 0.2% DMSO incubation in cell culture medium. Before extraction, the proteins were precipitated by addition of 500 μL cold methanol. After 30 min at –20°C, the mixture was centrifuged at 10 000 rpm for 5 min. Then 3 mL of water were added to the supernatant and the resulting solution was purified with a SepPack C18 cartridge (Waters Corp., Milford, MA, USA). The samples were eluted with 1 mL acetonitrile. This extract was reduced to dryness by speed-vac concentration (Savant SC-200, Savant Instruments, Farmingdale, NY, USA) and then dissolved in the HPLC solvent (50% 10 mM ammonium acetate in water, 50% acetonitrile).

HPLC/MS/MS analysis

HPLC/MS/MS analyses were performed on an Esquire ion trap mass spectrometer (Bruker Daltonics GmbH, Bremen, Germany) with an electrospray ionization source operating in alternative positive/negative ionization mode, coupled with a 150 × 2.1 mm C18 Kromasil column (Interchim). The flow rate was 0.2 mL/min of a 25 min linear gradient ranging from 10 to 80% B (90% acetonitrile in water) into A (90% 10 mM ammonium acetate in water, 10% acetonitrile). Electrospray ionization was performed at 4.5 kV and the capillary temperature was set to 310°C using a nitrogen gas flow rate of 40 mL/min and an auxiliary gas flow of 10 mL/min. Collision-induced dissociation of the selected precursor ion was carried out in the presence of helium with a collision

voltage of 0.70 V using a trap isolation width of 3 m/z units in order to isolate the whole isotopic cluster. Optimizations of MS and MSⁿ signals were carried out using infusion of commercial STG. Injections were performed in 50:50 acetonitrile/water containing 10 mM ammonium acetate. Data acquisition and reprocessing were performed using Bruker Daltonics Data Analysis 3.2 software.

The SMARTCyp 1.5.3 program^[25] was used to predict CYP metabolism. Mass Frontier 7.0 Spectral Interpretation Software (Thermo Fisher Scientific, San Jose, CA, USA) was used to simulate MS fragmentation in positive ion mode.

RESULTS AND DISCUSSION

Uniform ¹³C enrichment in STG

Before its use in metabolic studies, we checked that ¹³C-enriched STG presented an isotopic cluster characteristic of 10% ¹³C enrichment. The MS spectrum in positive ion mode contained the [M+H]⁺ ion at m/z 325 and also M+1 (m/z 326), M+2 (m/z 327), M+3 (m/z 328), M+4 (m/z 329) and M+5 (m/z 330) ions (Fig. 1). These peaks are in a 0.50:1.00:0.93:0.57:0.23:0.02 ratio relative to the most intense

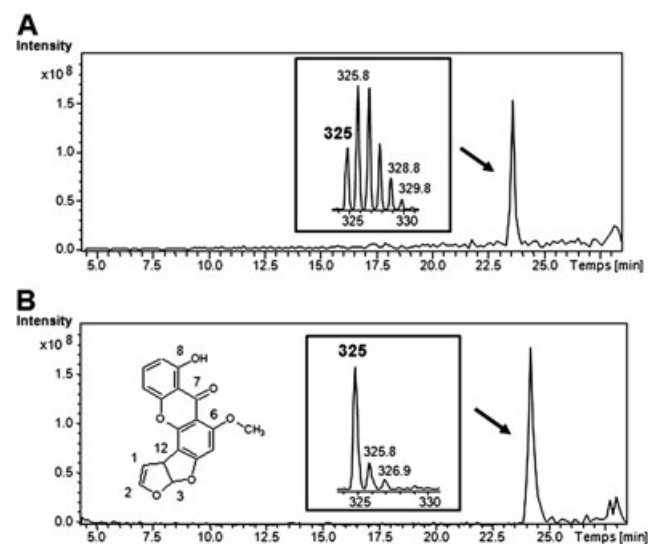


Figure 1. Representative HPLC/MS chromatograms and isotopic cluster of uniformly ¹³C-enriched sterigmatocystin after 10% ¹³C enrichment (A) and natural ¹²C sterigmatocystin with its structure (B).

ion at m/z 326, and the isotopic ratios are close to those expected from theoretical evaluation for 10% ¹³C enrichment.^[17] The accuracy on the obtained isotope pattern determined for 15 different patterns was inferior to 20% for each peak.

Metabolite studies by human P450 enzymes

In order to avoid the use of human microsomes which contain around 20 different CYP isoforms, we used recombinant human CYP which are obtained from insect cells transfected with only a specific CYP cDNA. A 30 min incubation of either commercial ¹²C or 10%-¹³C STG with 0.2 μ M recombinant human CYP1A1 enzyme in the presence of NADPH led to the significant formation of two metabolites M1 and M2. These metabolites, having retention time (t_R) = 19.5 min (M1) and 15.5 min (M2), were more polar than STG (t_R = 24.8 min). They had a molecular weight difference of one (+16 Da) (MW = 340) or two oxygen atoms (+32 Da) (MW = 356), respectively, relative to STG (Table 1). The metabolites formed using 10%-¹³C STG exhibited isotopic clusters similar to that of STG. Their [M+1]⁺ and [M+2]⁺ ions were more intense than [M]⁺. Collision-induced fragmentation of protonated M1 led to product ions at m/z 313, 299, 285, and 271 in positive ion mode (Fig. 2). Incubations in the presence of GSH led to the formation of a third metabolite (M3) having t_R = 11 min and an ion at m/z 644 in negative ion mode, which corresponds to an increase of 321 Da relative to the molecular weight of STG (Table 1). Incubations of STG with other human CYP isoforms led to the formation of M1 and M3 for CYP1A2, M3 for CYP3A4, and no metabolism for CYP2A6 and CYP2A13. All the metabolites exhibited an isotopic cluster profile in their mass spectra (Fig. 2), which confirmed their connection to STG. The MS² product ions also conserved an isotopic cluster (Fig. 2).

Based on its mass, its polarity and the monooxygenase activity of CYP, M1 was assigned as monohydroxysterigmatocystin (Table 1). CYP metabolism prediction using the SMARTCyp program indicates that two sites (C3 and C12) in STG bear a labile proton for CYP hydroxylation. The fragmentation pathway of protonated M1 in positive ion mode is in accordance with hydroxylation on C12 (Fig. 2). Interestingly, it had been demonstrated that CYP1A2 oxidizes aflatoxin B₁ in the same position, leading to aflatoxin M₁.^[26]

M3 was assigned as a GSH adduct of an oxidized metabolite (Table 1). This oxidation may occur in the 1-2-position since the STG-glutathione adduct formed on STG 1,2-oxide has been observed.^[27] Other possible structures include oxidation of the phenol ring to a reactive quinone which can then form a glutathione adduct.^[28] The detection

Table 1. Molecular weights (MW) for the compounds described in this study

	MW (Da)	Detection (m/z)
Sterigmatocystin	324	Positive and negative ion mode (325 ⁺ , 323 ⁻)
M1: monohydroxysterigmatocystin	340	Positive and negative ion mode (341 ⁺ , 339 ⁻)
M2: dihydroxysterigmatocystin	356	Negative ion mode (355 ⁻)
M3: GSH adduct of an oxidized metabolite	645	Negative ion mode (644 ⁻)
M4: sterigmatocystin-glucuro-conjugate	500	Negative ion mode (499 ⁻)
M5: sulfo-conjugate of monohydroxysterigmatocystin	420	Negative ion mode (419 ⁻)
M6: glucuro-conjugate of monohydroxysterigmatocystin	516	Negative ion mode (515 ⁻)

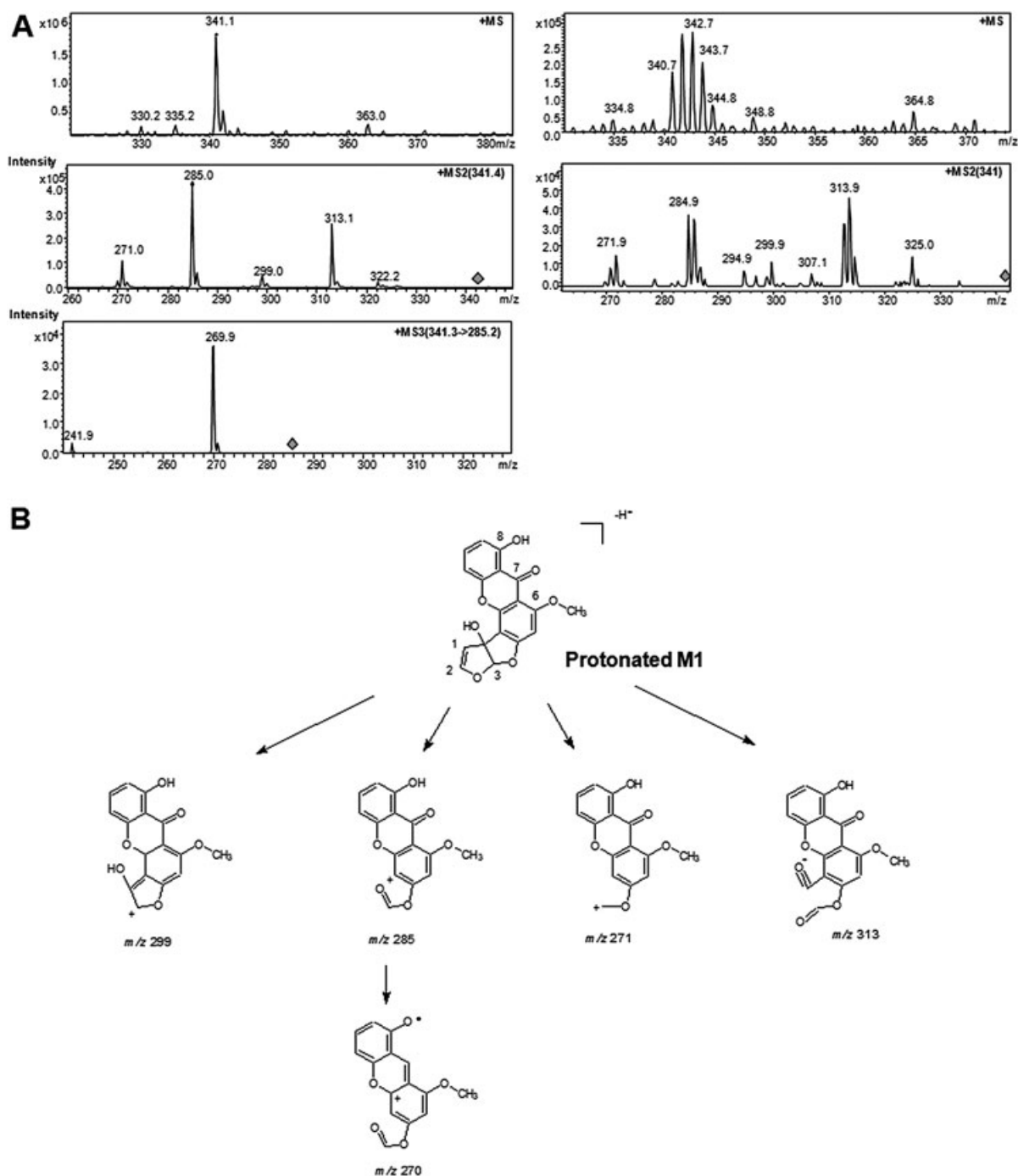


Figure 2. Representative HPLC/MS, MS² and MS³ chromatograms of the ion at *m/z* 341 in positive ionization mode (M+16, metabolite M1); with natural ¹²C sterigmatocystin on the left and with ¹³C-enriched sterigmatocystin on the right (A). Proposed fragmentation pathways of protonated-M1 in positive ion mode (B).

of a glutathione adduct after *in vitro* metabolism through CYP1A and CYP3A4 activities strongly suggests the formation of a transient reactive epoxide of STG, as has been described using either human hepatic microsomes or the Ames test.^[27,29] Such metabolic pathways have been described for aflatoxin B₁ hepatotoxicity.^[30] These mutagenic properties could be related to the pulmonary carcinomas described in mice after oral administration of STG.^[15,31]

M2 was assigned as dihydroxysterigmatocystin (Table 1). M2 may be formed by hydrolysis of M3 or by M1 hydroxylation. CYP metabolism prediction using the SMARTCyp program indicates that C3 is the most probable site for CYP hydroxylation in monohydroxysterigmatocystin (M1). Thus, M2 could also be a catechol or a *para*-diphenol on the phenyl ring, and it may be metabolized to a quinone.

STG metabolism in PTECs

After 48 h incubation with PTECs, only one STG metabolite, M4, was formed and this was identified as a glucuro conjugate. M4 was more polar than STG (at t_R = 11.5 min) and had an isotopic cluster shifted to M+176 relative to STG (MW = 500) (Fig. 3, Table 1). Fragmentation of deprotonated M4 in negative ion mode led to the formation of the MS cluster of deprotonated STG. The glucuronide formation was confirmed after hydrolysis with β -glucuronidase (data not shown). Only glucuronide conjugation with the free hydroxyl function on C8 is compatible with the mass of M4. Glucuronidation has been involved in metabolism of STG, and a STG glucuronide was detected in the urine of rat or monkey.^[32,33] Glucuronidation has been identified as an important pathway in the respiratory detoxification of environmental carcinogens such as polycyclic aromatic hydrocarbons.^[34]

After 24 h exposure of PTECs to 25 μ M β -naphthoflavone, 48 h STG incubation led to the formation of two new polar metabolites, M5 and M6, detected in negative ion mode. These metabolites had t_R values of 12.5 and 5.5 min and an isotopic cluster shifted to M+96 (MW = 420) and M+192 (MW = 516), respectively, relative to STG (Fig. 3). M5 was identified as a sulfo-conjugate (M+16+80) and M6 as a glucuro-conjugate (M+16+176) of monohydroxysterigmatocystin (Table 1). The product ions of deprotonated M5 and M6 show an M+16 isotopic cluster, which could correspond to a monohydroxysterigmatocystin product (M1 or hydroxylation on another location). Therefore, CYP1A1 metabolism may result in M1 formation, with rapid transformation into M5 and M6. Evidence for the epoxide formation and the subsequent formation of dihydroxysterigmatocystin (M2) or the glutathione conjugate (M3) was not found in porcine primary cells.

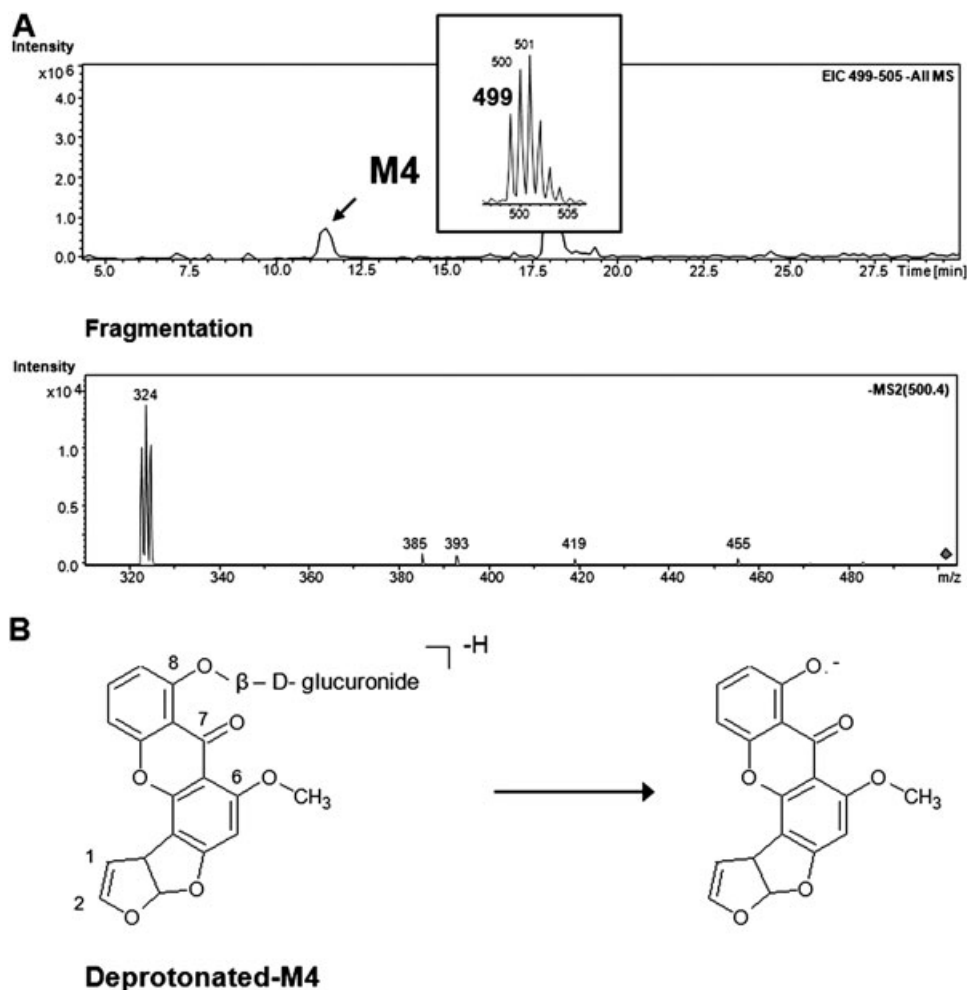


Figure 3. Representative HPLC/MS/MS chromatograms showing formation of sterigmatocystin metabolite in PTEC exposed to 1 μ M sterigmatocystin through the basal side of cultures (A): m/z 499 in negative ionization mode (M4) and MS² spectrum of M4 metabolite. Proposed fragmentation pathways of deprotonated M4 in negative ion mode (B).

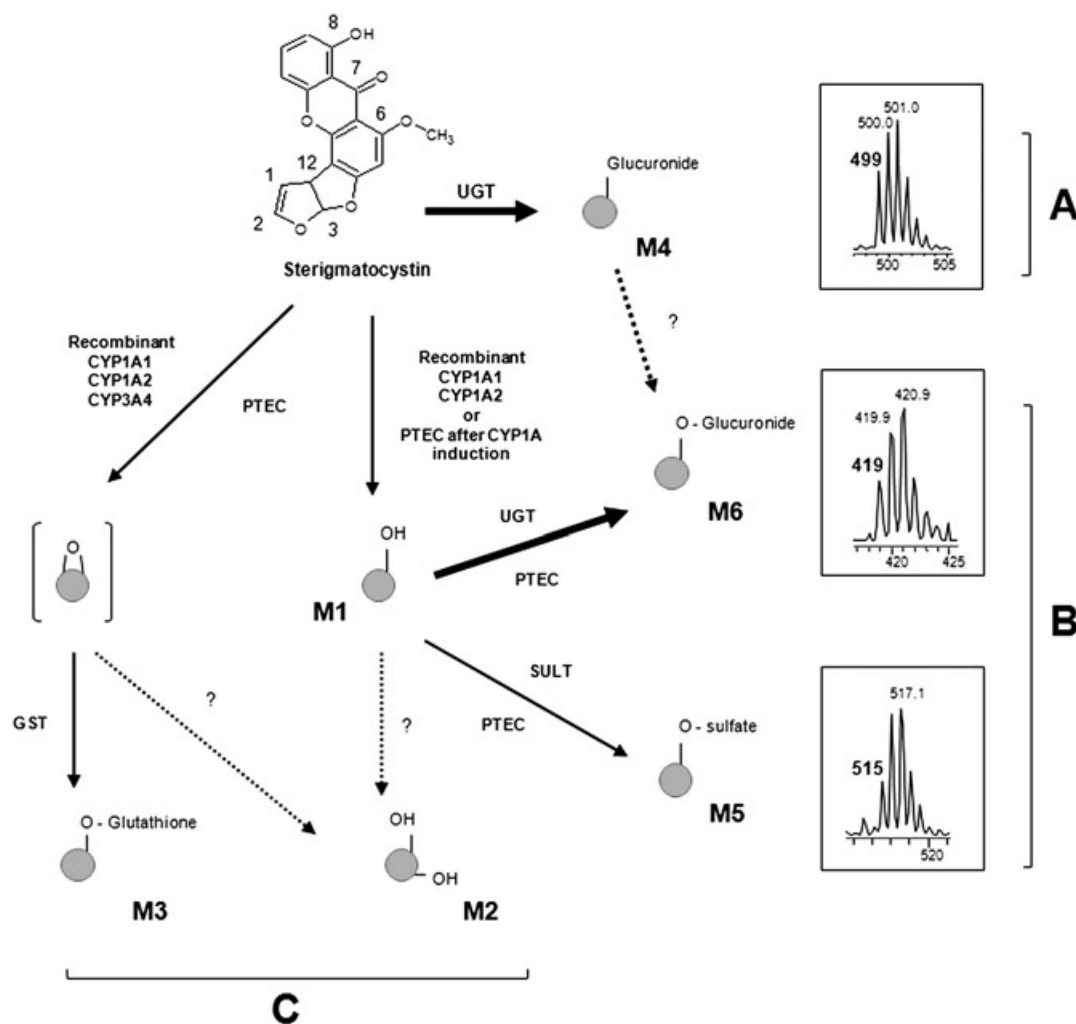


Figure 4. Putative pulmonary sterigmatocystin metabolism. Pathway A (top) indicates formation of a sterigmatocystin-glucuro conjugate (metabolite M4) in 'normal' airway epithelial cell cultures. Pathway B indicates formation of a sulfo conjugate and a glucuro conjugate of monohydroxysterigmatocystin (metabolites M5 and M6, respectively) in 'CYP1A1-induced' airway epithelium cultures. CYP1A1 amounts were increased by 24 h exposure to 25 μ M β -naphthoflavone. For high levels of CYP1A1 and in the presence of glutathione-S-transferases and glutathione, transient epoxide formation led to the glutathione conjugate or to dihydrodiol (pathway C, bottom).

CONCLUSIONS

Our study shows that STG is metabolized in porcine epithelial airway cells through phase 1 and 2 metabolism (Fig. 4, Table 1). This is the first study to report metabolite formation and detoxification pathways in the pulmonary stage using primary cells at an air-liquid interface. This metabolism seems close to that of aflatoxin B₁ with the formation of oxidized metabolites, a glutathione adduct and glucuronides. However, further studies are necessary to precisely locate the sites of oxidation. STG is mainly detoxified through glucuronidation (glucuronidation of STG and monohydroxysterigmatocystin). We also suggest that the metabolism of STG in the porcine airway tract *in vitro* is unable to produce significant amounts of reactive epoxide metabolites.

Finally, the uniformly enriched molecules with stable isotopes are very powerful tools for metabolism studies. Since the isotopic cluster signature is preserved for all the enriched metabolites, mass spectrometric analysis is easier than the classical approach.

Acknowledgements

We thank Sandrine Blondel and Anaïs Roussel for technical assistance. These studies were supported in part by AFSSET (Grant Numbers EST-2007-63 and EST-2008-27). We thank Andrew Lilley for English correction.

REFERENCES

- [1] M. A. Peyronneau, M. Delaforge, R. Riviere, J. P. Renaud, D. Mansuy. High affinity of ergopeptides for cytochromes P450 3A. Importance of their peptide moiety for P450 recognition and hydroxylation of bromocriptine. *Eur. J. Biochem.* **1994**, *223*, 947.
- [2] E. M. Isin, F. P. Guengerich. Complex reactions catalyzed by cytochrome P450 enzymes. *Biochim. Biophys. Acta* **2007**, *1770*, 314.
- [3] J. W. Bennett, M. Klich. Mycotoxins. *Clin. Microbiol. Rev.* **2003**, *16*, 497.

- [4] E. Bloom, L. F. Grimsley, C. Pehrson, J. Lewis, L. Larsson. Molds and mycotoxins in dust from water-damaged homes in New Orleans after hurricane Katrina. *Indoor Air* **2009**, *19*, 153.
- [5] J. Bungler, G. Westphal, A. Monnich, B. Hinnendahl, E. Hallier, M. Muller. Cytotoxicity of occupationally and environmentally relevant mycotoxins. *Toxicology* **2004**, *202*, 199.
- [6] K. Khoufache, O. Puel, N. Loiseau, M. Delaforge, D. Rivollet, A. Coste, C. Cordonnier, E. Escudier, F. Botterel, S. Bretagne. Verruculogen associated with *Aspergillus fumigatus* hyphae and conidia modifies the electrophysiological properties of human nasal epithelial cells. *BMC Microbiol.* **2007**, *7*, 5.
- [7] W. G. Sorenson, D. G. Frazer, B. B. Jarvis, J. Simpson, V. A. Robinson. Trichothecene mycotoxins in aerosolized conidia of *Stachybotrys atra*. *Appl. Environ. Microbiol.* **1987**, *53*, 1370.
- [8] S. Engelhart, A. Looock, D. Skutlarek, H. Sagunski, A. Lommel, H. Farber, M. Exner. Occurrence of toxigenic *Aspergillus versicolor* isolates and sterigmatocystin in carpet dust from damp indoor environments. *Appl. Environ. Microbiol.* **2002**, *68*, 3886.
- [9] T. Tuomi, K. Rejjula, T. Johnsson, K. Hemminki, E. L. Hintikka, O. Lindroos, S. Kalso, P. Koukila-Kahkola, H. Mussalo-Rauhamaa, T. Haahtela. Mycotoxins in crude building materials from water-damaged buildings. *Appl. Environ. Microbiol.* **2000**, *66*, 1899.
- [10] K. F. Nielsen, S. Gravesen, P. A. Nielsen, B. Andersen, U. Thrane, J. C. Frisvad. Production of mycotoxins on artificially and naturally infested building materials. *Mycopathologia* **1999**, *145*, 43.
- [11] K. Yabe, H. Nakajima. Enzyme reactions and genes in aflatoxin biosynthesis. *Appl. Microbiol. Biotechnol.* **2004**, *64*, 745.
- [12] R. H. Adamson. Induction of hepatocellular carcinoma in nonhuman primates by chemical carcinogens. *Cancer Detect. Prev.* **1989**, *14*, 215.
- [13] K. Fujii, H. Kurata, S. Odashima, Y. Hatsuda. Tumor induction by a single subcutaneous injection of sterigmatocystin in newborn mice. *Cancer Res.* **1976**, *36*, 1615.
- [14] M. Mabuchi. Sequential hepatic changes during sterigmatocystin-induced carcinogenesis in the rat. *Jpn J. Exp. Med.* **1979**, *49*, 365.
- [15] I.A.R.C. Sterigmatocystin. *IARC Monogr. Eval. Carcinog. Risk Chem. Man.* **1976**, *10*, 245.
- [16] I. R. McConnell, R. C. Garner. DNA adducts of aflatoxins, sterigmatocystin and other mycotoxins. *IARC Sci. Publ.* **1994**, *49*.
- [17] F. Bravin, R. C. Duca, N. Loiseau, M. Pean, O. Puel, M. Delaforge. Production and use of mycotoxins uniformly enriched with stable isotopes for their dosage in biological samples. *World Mycotoxins J.* **2008**, *1*, 275.
- [18] F. P. Guengerich, in *Human Cytochrome P450 Enzymes*, (Ed: P. R. Ortiz de Montellano), Plenum, New York, **2005**.
- [19] C. L. Crespi, B. W. Penman, et al. Human cytochrome P450IIA3: cDNA sequence, role of the enzyme in the metabolic activation of promutagens, comparison to nitrosamine activation by human cytochrome P450III1. *Carcinogenesis* **1990**, *11*, 1293.
- [20] X. Y. He, L. Tang, S. L. Wang, Q. S. Cai, J. S. Wang, J. Y. Hong. Efficient activation of aflatoxin B1 by cytochrome P450 2A13, an enzyme predominantly expressed in human respiratory tract. *Int. J. Cancer* **2006**, *118*, 2665.
- [21] J. D. Kelly, D. L. Eaton, F. P. Guengerich, R. A. Coulombe Jr. Aflatoxin B1 activation in human lung. *Toxicol. Appl. Pharmacol.* **1997**, *144*, 88.
- [22] T. R. Van Vleet, P. J. Klein, R. A. Coulombe Jr. Metabolism of aflatoxin B1 by normal human bronchial epithelial cells. *J. Toxicol. Environ. Health A* **2001**, *63*, 525.
- [23] O. Cabaret, O. Puel, F. Botterel, M. Pean, K. Khoufache, J. M. Costa, M. Delaforge, S. Bretagne. Metabolic detoxication pathways for sterigmatocystin in primary tracheal epithelial cells. *Chem. Res. Toxicol.* **2010**, *23*, 1673.
- [24] K. Khoufache, O. Cabaret, C. Farrugia, D. Rivollet, A. Alliot, E. Allaire, C. Cordonnier, S. Bretagne, F. Botterel. Primary *in vitro* culture of porcine tracheal epithelial cells in an air-liquid interface as a model to study airway epithelium and *Aspergillus fumigatus* interactions. *Med. Mycol.* **2010**, *48*, 1049.
- [25] Available: <http://www.farma.ku.dk/smartyyp/>
- [26] F. P. Guengerich, W. W. Johnson, T. Shimada, Y. F. Ueng, H. Yamazaki, S. Langouet. Activation and detoxication of aflatoxin B1. *Mutat. Res.* **1998**, *402*, 121.
- [27] K. D. Raney, T. Shimada, D. H. Kim, J. D. Groopman, T. M. Harris, F. P. Guengerich. Oxidation of aflatoxins and sterigmatocystin by human liver microsomes: significance of aflatoxin Q1 as a detoxication product of aflatoxin B1. *Chem. Res. Toxicol.* **1992**, *5*, 202.
- [28] E. S. Krol, J. L. Bolton. Oxidation of 4-alkylphenols and catechols by tyrosinase: ortho-substituents alter the mechanism of quinoid formation. *Chem. Biol. Interact.* **1997**, *104*, 11.
- [29] T. Shimada, C. L. Hayes, H. Yamazaki, S. Amin, S. S. Hecht, F. P. Guengerich, T. R. Sutter. Activation of chemically diverse procarcinogens by human cytochrome P-450 1B1. *Cancer Res.* **1996**, *56*, 2979.
- [30] J. S. Wang, J. D. Groopman. DNA damage by mycotoxins. *Mutat. Res.* **1999**, *424*, 167.
- [31] G. M. Zwicker, W. W. Carlton, J. Tuite. Long-term administration of sterigmatocystin and *Penicillium viridicatum* to mice. *Food Cosmet. Toxicol.* **1974**, *12*, 491.
- [32] J. J. Olson, F. S. Chu. Immunochemical Studies of Urinary Metabolites of Sterigmatocystin in Rats. *J. Agric. Food Chem.* **1993**, *41*, 250.
- [33] P. G. Thiel, M. Steyn. Urinary excretion of the mycotoxin, sterigmatocystin by vervet monkeys. *Biochem. Pharmacol.* **1973**, *22*, 3267.
- [34] R. W. Dellinger, J. L. Fang, G. Chen, R. Weinberg, P. Lazarus. Importance of UDP-glucuronosyltransferase 1A10 (UGT1A10) in the detoxification of polycyclic aromatic hydrocarbons: decreased glucuronidative activity of the UGT1A10139Lys isoform. *Drug Metab. Dispos.* **2006**, *34*, 943.