PERGAMON

Phytochemistry 53 (2000) 377–378

PHYTOCHEMISTRY

www.elsevier.com/locate/phytochem

Uptake of radiolabelled ochratoxin A from soil by coffee plants

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Received 23 June 1999; received in revised form 28 September 1999; accepted 7 October 1999

Abstract

[^{3}H, ^{14}C] Ochratoxin A, prepared biosynthetically, was applied in dilute $NaHCO_3$ solution to the soil in which coffee plants had grown to four pairs of leaves. Three weeks later the compound, isolated from dilute $NaHCO_3$ extract of leaves by immunoaffinity chromatography, was detected by scintillation counting as a 1–2 ppm component of leaf dry weight, greatly exceeding the trace (ppb) occurrence of ochratoxin A in some green coffees, which therefore might arise in the field directly from fungal activity in soil rather than from fungal infection of cherries or processed green coffee. © 2000 Elsevier Science Ltd. All rights reserved.

Keywords: Ochratoxin A; *Coffea arabica*; Immunoaffinity; Radioactivity

1. Introduction

Increasingly sensitive analytical methodology involving the use of immunoaffinity columns has enabled specific recognition of trace (ppb) amounts of ochratoxin A rather widely in agricultural commodities and products. The range of commodities has recently included coffee beans. As with very occasional higher incidence in coffee, the sole source of ochratoxin A has been assumed to be mould infection of cherries and/or processed green coffee, but it was recently proposed that trace amounts might arise by direct uptake of the toxin from soil (Mantle, 1998), where the principal biosynthetic source in the tropics, *Aspergillus ochraceus*, is often endemic (Frank, 1999). In temperate latitudes, the principal source of ochratoxin A is *Penicillium verrucosum*. A precedent for direct uptake from soil is the uptake of the mycotoxin verruculogen by intact root systems of monocotyledonous and dicotyledonous plants (Day & Mantle, 1990). The feasibility of similar uptake into coffee plants has been tested experimentally using ochratoxin A radiolabelled with ^{3}H and ^{14}C.

2. Results and discussion

^{3}H radioactivity measured in immunoaffinity column eluate from leaf tissue of coffee plants given radioactive ochratoxin A was 350 dpm above background (^{3}H : ^{14}C ratio 5:1), equivalent to 1.3 μg ochratoxin A (estimated 1–2 ppm in dry plant tissue). Selectivity of ochratoxin A by immunoaffinity technology is highly specific, there being even low cross-reactivity with the des-chloro analogue ochratoxin B. Eluate from a similar mass of control plant tissue, given bicarbonate solution only, gave only background radioactivity. Since the root system of all plants had been undisturbed since planting the germinated seeds, it is concluded that uptake of ochratoxin A from soil by coffee plants to create a sampled bean composition of the trace amounts found quite frequently (about 1 ppb) is feasible. The extent to which this occurs naturally under field conditions for coffee remains to be demonstrated, but a similar mechanism might also apply to trace

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PII: S0031-9422(99)00527-0

amounts of ochratoxin A found in commercial commodities from other agricultural crops.

3. Experimental

3.1. Plants

Seeds of *Coffea arabica* var. Mundo Novo cultivar LCP 388-17 were sown on 23 June 1998 at the Instituto Biologico de Sao Paulo, Brazil and seedlings in the 'fosforito' stage were provided in September 1998, via Illycaffe, Trieste, Italy for cultivation in London in peat-based compost in plastic pots. After six months at 18–20°C in natural daylight supplemented by a mercury vapour lamp, coffee plants had four pairs of expanded leaves.

3.2. Radiolabelled ochratoxin A

Spores of *A. ochraceus* (isolate D2306, Harris, 1996) were inoculated on to 40 g sterile shredded wheat moistened with H_2O (16 ml) and incubated in shaken solid substrate fermentation in a 500 ml Erlenmeyer flask at 29°C. Five days later, L[2,3,4,5,6 ^{3}H] phenylalanine (100 μCi) and sodium[1-^{14}C] acetate (3.5 μCi) were added in 1.5 ml H_2O as a fine spray over the substrate. On day 7, a similar amount of [^{3}H] phenylalanine, together with L[U-^{14}C] phenylalanine (30 μCi), was given similarly. The flask contents were extracted on day 10 with EtOAc/0.01 M H_3PO_4 (9:1, 300 ml) and the filtrate extracted exhaustively with 1% aqueous $NaHCO_3$. The aqueous phase was adjusted to pH 6 with HCl, precipitating ochratoxins A and B which were then extracted exhaustively with EtOAc. The extract was chromatographed preparatively over silica gel (Macherey-Nagel SIL G-200 UV_{254}; solvent: EtOAc/toluene/HCOOH [15:4:1]) and the chromatogram autoradiographed to demonstrate exclusive coincidence of ^{14}C only with ochratoxins A and B (detected by fluorescence at 350 nm), which were eluted from silica gel with propanol-2-ol. The yield of radiolabelled ochratoxin A from shredded wheat was approximately 1 mg g^{-1}. [^{3}H, ^{14}C] ochratoxin A (20 mg; specific activity ^{3}H 270 dpm μg^{-1} [ratio ^{3}H : ^{14}C 6:1, measured by scintillation counter in a Packard Tri-Carb 2200 CA at an order of radioactivity similar to that subsequently measured in coffee plant extract]) was dissolved in 0.1 M $NaHCO_3$ (50 ml) and given to a pot containing three coffee plants by capillarity uptake from the bottom.

3.3. Ochratoxin A analysis of plant tissue

Three weeks after administration of [^{3}H, ^{14}C] ochratoxin A to the soil of coffee plants, the tissues above each pair of expanded cotyledons (total 2.7 g fresh weight) were excised, ground in a mortar in 0.1 M $NaHCO_3$, filtered through a glass sinter and the extract (20 ml) mixed with phosphate buffered saline (PBS, 10 ml). The solution was passed through an immunoaffinity column (Ochraprep, Rhone-Diagnostics Technologies). The column was washed with PBS (3 ml) and then with MeOH : H_2O (1:4, 1 ml), and ochratoxin A eluted with MeOH : CH_3COOH (98:2, 1.5 ml) into a scintillation vial for radioactivity measurement.

Acknowledgements

I thank Dr. E. Illy and Dr. M. Petracco, Illycaffe, Trieste for helpful discussion and for providing coffee seedlings. Gift of Ochraprep immunoaffinity columns by Rhone-Diagnostics Technologies is gratefully acknowledged.

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