Biotransformation of quinoxaline by *Streptomyces badius*

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J.B. SUTHERLAND, F.E. EVANS, J.P. FREEMAN AND A.J. WILLIAMS. 1996. Quinoxaline, a mutagenic azaarene produced in foods during cooking, was added to cultures of *Streptomyces badius* ATCC 39117. After 24 h, the cultures were extracted with ethyl acetate. Two major metabolites were purified by liquid chromatography and identified by mass spectrometry and nuclear magnetic resonance spectroscopy as 3,4-dihydro-2(1H)-quinoxalinone and 2(1H)-quinoxalinone.

**INTRODUCTION**

Quinoxaline (1,4-diazanaphthalene) is a heterocyclic compound produced during cooking of various foods (Kinlin et al. 1972; Mussinan and Walradt 1974; Aeschbacher et al. 1989). It is weakly mutagenic in *Salmonella typhimurium* strain TA98 (Aeschbacher et al. 1989) and inhibits the growth of some ciliate protozoa (Schultz and Cajina-Quezada 1982) and plant-pathogenic fungi (Izumi et al. 1988).

Little is known about the metabolism of quinoxaline except that *Pseudomonas putida* metabolizes it to quinoxaline cis-5,6-dihydrodiol, 5-hydroxyquinoxaline, and 2(1H)-quinoxaline (Boyd et al. 1987, 1993). We have found that *Streptomyces badius* has the ability to cometabolize the heterocyclic ring of quinoxaline.

**MATERIALS AND METHODS**

Cultures of *S. badius* strain 252 (Crawford and Sutherland 1979) (=ATCC 39117) were grown at 37°C on a rotary shaker in cotton-plugged 2-l Erlenmeyer flasks, each containing 500 ml of tryptone yeast extract broth (3.0 g l⁻¹ Bacto-tryptone [Difco Laboratories, Detroit, MI, USA], 3.0 g l⁻¹ Bacto-yeast extract and 1.0 g l⁻¹ K₂HPO₄, pH 7.4). After 48 h, each culture was dosed aseptically with 1.0 ml of filter-sterilized N,N-dimethylformamide containing 150 mg ml⁻¹ quinoxaline (99%, Janssen Chimica, Beerse, Belgium); the final quinoxaline concentration was 2.3 mmol l⁻¹. Noninoculated control flasks and cultures without quinoxaline were also prepared.

After incubation for another 24 h, the cultures and controls were extracted with equal volumes of ethyl acetate. The solvent was dried over anhydrous sodium sulphate and evaporated in vacuo.

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Fig. 1 HPLC chromatogram of the ethyl acetate extract of *Streptomyces badius* incubated with quinoxaline. I, Metabolite I; II, metabolite II

for metabolite I (Fig. 2) contains an apparent molecular ion [M$^+$] at m/z 148 and fragment ions at m/z 119 (M$^+$-HCO), 92 (M$^+$-NHCOCH), 65, 60 and 52. Although no spectral library matches were obtained for this spectrum, the major fragment ions are consistent with losses from the heterocyclic ring of 3,4-dihydro-2(1H)-quinoxalinone.

Table 1 Proton NMR spectral parameters for 3,4-dihydro-2(1H)-quinoxalinone and 2(1H)-quinoxalinone, two metabolites produced from quinoxaline by *Streptomyces badius*

| Assignment | Chemical shifts (δ, in ppm) |
|------------|-----------------------------|
| Metabolite I | Metabolite II |
| 3,4-dihydro-2(1H)-quinoxalinone | 2(1H)-quinoxalinone |
| 1 | 9.15 | 11.13 |
| 3 | 3.80† | 8.13 |
| 4 | 5.23 | — |
| 5 | 6.73 | 7.99 |
| 6 | 6.79 | 7.32 |
| 7 | 6.65 | 7.56 |
| 8 | 6.84 | 7.39 |

*Coupling constants (in Hz) were as follows: for metabolite I, J$_{4,5}$ = 1.9; J$_{4,6}$ = 8.2; J$_{5,6}$ = 1.5; J$_{6,7}$ = 7.3; J$_{8,9}$ = 1.3; and J$_{7,8}$ = 8.2; for metabolite II, J$_{4,5}$ = J$_{6,7}$ = J$_{8,9}$ = 7.7; J$_{5,6}$ = 1.5; and J$_{7,8}$ = 1.3. In addition, for metabolite I, J$_{1,3}$ and J$_{1,5}$ were detected as resonance broadening.

†Two equivalent protons (3ab).

The NMR spectrum of metabolite I in acetone-d$_6$ (Table 1) showed eight major resonances, two of which were equivalent and located in the aliphatic region (H3ab, 3.80 ppm). Two resonances exhibited broadening, which is characteristic of exchangeable protons (NH4, 5.23 ppm and NH1, 9.15 ppm). Homonuclear decoupling experiments established that the H3ab resonances were coupled to NH4. Saturation of the NH4 resonance resulted in a nuclear Overhauser effect (NOE) to the H3ab resonances and to a doublet in the aromatic region (H5, 6.73 ppm). Saturation of the NH1 resonance resulted in an NOE to another doublet (H8, 6.84 ppm). These experiments and additional homonuclear decoupling experiments enabled the assignment of all of the resonances in 3,4-dihydro-2(1H)-quinoxalinone.

Metabolite II coeluted on the HPLC with authentic 2(1H)-quinoxalinone (= 2-quinoxalinol, Aldrich Chemical Co.,

Fig. 2 Electron ionization mass spectrum and structure of metabolite I, 3,4-dihydro-2(1H)-quinoxalinone, produced by *Streptomyces badius*
Milwaukee, WI, USA). It had a u.v./visible absorption spectrum, which was identical to that of the 2(1H)-quinoxalinone standard, with λmax values at 202, 230, 280 and 345 nm. The mass spectrum obtained from metabolite II contained an apparent molecular ion [M'] at m/z 146 and major fragment ions at m/z 118, 91, 64 and 63. It was identical to that of 2(1H)-quinoxalinone (Stubbley et al. 1979).

The NMR spectrum of metabolite II (Table 1) was also the same as that of authentic 2(1H)-quinoxalinone. The NMR spectral assignments are based in part on the observation of an NOE to the doublet at 7.39 ppm (H8) resulting from saturation of the NH1 resonance.

**DISCUSSION**

Metabolite I was shown to be 3,4-dihydro-2(1H)-quinoxalinone, a lactam form that predominated over 3,4-dihydro-2-hydroxyquinoxaline, the lactin form. The mechanism of formation by S. badius is unknown but may involve a hydratase-catalysed addition of water.

Metabolite II was shown to be 2(1H)-quinoxalinone, a lactam form that predominated over 2-hydroxyquinoxaline, the lactin form. The NMR experiments confirmed previous arguments based on chemical shifts (Milch and Horváth 1970). This metabolite is also produced by Ps. putida (Boyd et al. 1987, 1993). Six other Streptomyces species were also tested and found to produce 2(1H)-quinoxalinone (data not shown) but none produced both metabolites. The formation of 2(1H)-quinoxalinone may be due to (a) the oxidation of 3,4-dihydro-2(1H)-quinoxalinone, (b) the nonenzymatic rearrangement of an aren ring oxide produced by a cytochrome P-450 monooxygenase (Trower et al. 1988; Sutherland et al. 1990), or (c) an enzyme similar to quinoline oxidoreductase (Peschke and Lingens 1991). Although the aldehyde oxidase from rabbit liver oxidizes quinoxaline to 2,3(1,4H)-quinoxalinedione (=2,3-dihydroxyquinoxaline) via 2(1H)-quinoxalinone (McCormack et al. 1978), no compound that coeluted with authentic 2,3-dihydroxyquinoxaline (Janssen Chimica) was detected in the extracts from cultures of S. badius.

Unlike Ps. putida (Boyd et al. 1987, 1993), S. badius did not metabolize the carbocyclic ring of quinoxaline. We conclude that S. badius transformed quinoxaline to 3,4-dihydro-2(1H)-quinoxalinone and 2(1H)-quinoxalinone by metabolism of the heterocyclic ring.

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