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Microbial metabolism of 1-aminoanthracene by *Beauveria bassiana*

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Abstract—The carcinogen and mutagen, 1-aminoanthracene, was efficiently metabolized by the fungal strain *Beauveria bassiana* ATCC 7159 to yield three new metabolites identified as 1-acetamido-5-[(4′-*O*-methyl-β-D-glucopyranosyl)oxy]anthracene, 1-acetamido-8-[(4′-*O*-methyl-β-D-glucopyranosyl)oxy]anthraquinone, and 1-acetamido-6-[(4′-*O*-methyl-β-D-glucopyranosyl)oxy]anthraquinone, together with 1-acetamidoanthracene and 1-acetamidoanthraquinone. Formation of these metabolites suggests that the metabolic pathways of 1-aminoanthracene in *B. bassiana* ATCC 7159 involve acetylation, oxidation, hydroxylation, and *O*-methylglucosylation.

1. Introduction

Aromatic amines are widely used in industry and many aniline derivatives are synthetic precursors to a variety of colorants routinely employed in the textile, paper, leather, plastic, cosmetic, pharmaceutical, and food industries.[1,2] Most aromatic amines are known to be mutagenic and carcinogenic especially after their metabolic activation.[3] The aromatic amine, 1-aminoanthracene (**1**), has been reported to be mutagenic[3] and to possess genotoxic potency in *Drosophila melanogaster*.[4] This compound could also be transformed into a direct-acting mutagen by the reaction with nitrites[5] frequently used in the preservation of meat products. Although bacterial biodegradation and biotransformation of polycyclic aromatic hydrocarbons (PAHs) such as anthracene and phenanthrene are known,[6–8] no biotransformation studies of 1-aminoanthracene have thus far been reported. In continuing our interest in biotransformation of aromatic compounds,[9–11] we have investigated the metabolism of 1-aminoanthracene by the fungal strain, *Beauveria bassiana* ATCC 7159. This strain has previously been used in the biotransformation of more than 300 different substrates[12] and has displayed a variety of enzymatic activities.[13–16] Biotransformation of 1-aminoanthracene resulted in five metabolites of which three were new compounds. Structural analysis of these metabolites suggested that biotransformation pathway of this substrate by *B. bassiana* involved acetylation, oxidation, hydroxylation, and *O*-methylglucosylation.

A probable sequence of these enzymatic reactions is proposed.

2. Results and discussion

Five metabolites of 1-aminoanthracene (**1**) were isolated from the biotransformation broth and freeze–dried mycelia of *B. bassiana* ATCC 7159. Two of the metabolites were identified as 1-acetamidoanthracene (**2**) and 1-acetamidoanthraquinone (**3**) on the basis of their mass and NMR spectroscopic data, and by comparison with synthetic samples obtained by acetylation of 1-aminoanthracene and 1-aminoanthraquinone. The third metabolite (**4**) was determined to have the molecular formula $C_{23}H_{25}NO_7$ by high-resolution ESI-MS and its NMR spectra indicated structural similarities to **2**. Compared to 1-acetamidoanthracene (**2**) it had an additional $C_7H_{13}O_6$ which was consistent with the presence of a 4-*O*-methylglucose moiety, a group which we have previously found *B. bassiana* ATCC 7159 was capable of introducing to aromatic substrates.[11] The ^{13}C NMR spectrum of this metabolite analyzed with the help of its DEPT spectrum showed the presence of 13 methines, one oxygenated methylene, two methyls, and seven

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quarternary carbon signals. It had an aromatic methine less and a quarternary carbon (δ 152.7) more than **2** (Table 2) suggesting that it was substituted by another group at an aromatic position. The presence of a 4-*O*-methylglucose moiety in this metabolite was confirmed by the occurrence of five methine carbon (CH) signals at δ 100.5, 73.6, 75.7, 79.0, and 76.1, one methylene carbon (CH_2) signal at δ 60.3 and one OCH_3 signal at δ 59.7 in its ^{13}C NMR spectrum. Two singlets at δ 8.70 and δ 8.97 in the ^1H NMR spectrum of this metabolite were assigned to H-9 and H-10, respectively. The remaining signals in its ^1H NMR spectrum (Table 1) analyzed with the help of COSY showed two similar spin-systems of three aromatic protons with coupling constants suggesting C-5 or C-8 to be the likely positions to which the 4-*O*-methylglucose moiety was introduced. The high *J* value of the anomeric proton H-1′ suggested the presence of a β-glycosidic linkage in **4**. The correlations of proton H-10 (δ 8.97) with C-4 (δ 125.5) and C-5 (δ 152.7) in the HMBC spectrum indicated that the 4-*O*-methylglucose is attached to C-5 through an oxygen. Based on the foregoing evidence, this metabolite was identified as 1-acetamido-5-[(4′-*O*-methyl-β-D-glucopyranosyl)oxy]anthracene (**4**).

Metabolite **5** exhibited a peak due to $[M+Na]^+$ at *m*/*z* 480.1270 in its high-resolution ESI-MS, suggesting a molecular formula of $C_{23}H_{23}NO_9$. Spectroscopic data for this compound were very similar to those of 1-acetamidoanthraquinone (**3**) and suggested that a 4-*O*-methylglucose moiety was introduced during the biotransformation. This was further confirmed by the presence of sugar signals at δ 101.2, 74.4, 77.2, 79.7, 76.7, 61.1, and 60.6 in its ^{13}C NMR spectrum (Table 2). The two carbonyl group signals at δ 187.1 and δ 181.8 confirmed that it is an anthraquinone. The HMBC correlations of H-6 to C-8 and C-10a as well as H-5 to C-10 and C-7 suggested that the newly introduced 4-*O*-methylglucose moiety is linked to the anthraquinone moiety at C-8 through an oxygen atom. Based on the detailed analysis of COSY, HSQC, and HMBC spectra, this biotransformation product was identified as 1-acetamido-8-[(4′-*O*-methyl-β-D-glucopyranosyl)oxy]anthraquinone (**5**).

Metabolite **6** had the same molecular formula as **5**. In its ^{13}C NMR spectrum (Table 2) two carbonyl signals at δ 185.5 and δ 181.8, the signals for $NHCOCH_3$ moiety at C-1, and the signals for the 4-*O*-methylglucose moiety were observed. In its ^1H and COSY NMR spectra, the spin-system for H-2, H-3, and H-4 was the same as that of **5**, while the second spin-system showed a different splitting pattern (d, dd, and d) with coupling constants of 8.8 and 2.4 Hz, from which it could be deduced that the 4-*O*-methylglucosyloxy moiety is located at C-6 or C-7. The HMBC correlations of H-8 to C-6 and C-10a confirmed the attachment of the sugar moiety to C-6 of **6**. Thus, this metabolite was identified as 1-acetamido-6-[(4′-*O*-methyl-β-D-glucopyranosyl)oxy]anthraquinone (**6**).

Biotransformation of 1-aminoanthracene (**1**) by *B. bassiana* ATCC 7159 resulted in three major (**4**–**6**) and two minor (**2** and **3**) metabolites. As the chemical acetylation of 1-aminoanthraquinone required more vigorous conditions than those required for 1-aminoanthracene (see Section 3, **3.8** and **3.9**), it is probable that during the microbial biotransformation, the acetylation reaction of 1-aminoanthracene (**1**) to provide 1-acetamidoanthracene (**2**) would have preceded the oxidation of it to 1-aminoanthraquinone (**3**). Feeding *B. bassiana*

Table 1. ^1H NMR (500 MHz) spectroscopic data for metabolites **4**–**6** (in DMSO-d_6)

Position	δ (multiplicities, *J*)		
	4	**5**	**6**
1-NH	10.4 (s)	11.9 (s)	12.1 (s)
2	7.75*	8.87 (d, 6.1)	8.96 (d, 8.1)
3	7.48 (t, 7.8)	7.86*	7.87 (t, 8.0)
4	7.91 (d, 8.4)	7.82*	7.93 (d, 7.0)
5		7.67 (d, 8.3)	7.66 (d, 2.4)
6	7.12 (d, 7.4)	7.84*	
7	7.43 (t, 8.0)	7.91 (d, 7.7)	7.54 (dd, 8.8, 2.4)
8	7.75*		8.21 (d, 8.8)
9	8.70 (s)		
10	8.97 (s)		
12	2.25 (s)	2.25 (s)	2.26 (s)
1′	5.13 (d, 7.1)	5.15 (d, 6.9)	5.18 (d, 7.7)
2′	3.53 (m)	3.43 (m)	3.32 (m)
3′	3.48 (m)	3.45 (m)	3.48 (m)
4′	3.13 (t, 9.0)	3.09 (t, 9.0)	3.08 (t, 9.2)
5′	3.52 (m)	3.48 (m)	3.51 (m)
6′	3.69 (dd, 11.2, 4.8)	3.64 (brd, 11.6)	3.64 (dd, 10.0, 5.5)
	3.56 (m)	3.53 (m)	3.54 (m)
4′-OCH_3	3.49 (s)	3.47 (s)	3.46 (s)

* Overlapping signals.

Table 2. ^{13}C NMR (125 MHz) spectroscopic data for metabolites **4**–**6** (in DMSO-d_6)

Position	δ, multiplicities		
	4	**5**	**6**
1	133.4, qC	141.6, qC	141.3, qC
2	120.2, CH	125.2, CH	125.4, CH
3	125.0, CH	136.7, CH	135.4, CH
4	125.5, CH	122.5, CH	121.7, CH
5	152.7, qC	123.1, CH	112.6, CH
6	106.8, CH	136.3, CH	161.9, qC
7	125.8, CH	121.6, CH	122.1, CH
8	121.6, CH	158.2, qC	129.7, CH
9	120.3, CH	187.1, qC	185.5, qC
9a	131.7, qC	115.1, qC	127.9, qC
9b	126.4, qC	118.0, qC	117.4, qC
10	121.4, CH	181.8, qC	181.8, qC
10a	124.3, qC	136.4, qC	134.2, qC
10b	131.3, qC	136.4, qC	133.9, qC
11	168.9, qC	170.3, qC	169.4, qC
12	13.7, CH_3	26.2, CH_3	25.3, CH_3
1′	100.5, CH	101.2, CH	99.7, CH
2′	73.6, CH	74.4, CH	73.3, CH
3′	75.7, CH	77.2, CH	75.9, CH
4′	79.0, CH	79.7, CH	78.7, CH
5′	76.1, CH	76.7, CH	75.7, CH
6′	60.3, CH_2	61.1, CH_2	60.0, CH_2
4′-OCH_3	59.7, CH_3	60.6, CH_3	59.6, CH_3

ATCC 7159 with synthetic 1-acetamidoanthracene (**2**) resulted in the isolation of metabolites **3**–**6** whereas feeding with synthetic 1-acetamidoanthraquinone (**3**) resulted in only metabolites **5** and **6** (Fig. 1). The above findings together with the difference in rates observed for the chemical acetylation of 1-aminoanthracene (**1**) and 1-aminoanthraquinone (**2**) suggested that the substrate, 1-aminoanthracene, was first acetylated giving 1-acetamidoanthracene which would then undergo further biotransformation by two different pathways (Fig. 1). The 4-*O*-methylglucose moiety can then be introduced to 1-acetamidoanthracene to form 1-acetamido-5-[(4′-*O*-methyl-β-D-glucopyranosyl)oxy]anthracene (**4**), involving hydroxylation of the aromatic carbon C-5 followed by 4-*O*-methylglucosylation of the newly introduced hydroxyl group. On the other hand, 1-acetamidoanthracene can be first oxidized to 1-acetamidoanthraquinone (**3**) followed by hydroxylation at C-6 or C-8 and 4-*O*-methylglucosylation of the newly introduced hydroxyl groups affording metabolites **5** and **6** (Fig. 1). The overall metabolism of 1-aminoanthracene by *B. bassiana* therefore appears to involve four different enzymes, acetylase, oxidase, hydroxylase, and 4-*O*-methyglucosyltransferase. This constitutes the first report of the microbial metabolism of 1-aminoanthracene (**1**).

Although in this study, we have not evaluated the mutagenicity of 1-aminoanthracene (**1**) and its biotransformation products **2**–**6**, Trieff et al. have previously reported that the mutagenicity of aromatic amines was enhanced with increasing lipophilicity and was reduced when the amino group was located *ortho* to the ring junction or present as an acetamido moiety.[17] Thus, it is possible that the biotransformation products **2**–**6** containing polar *O*-methylglucosyl moieties and carrying acetamido groups may possess decreased mutagenicity compared with the substrate, 1-aminoanthracene (**1**), and that the metabolism of **1** by *B. bassiana* may be carried out for the detoxification of this mutagen.

3. Experimental

3.1. General methods

Melting points were determined with a Gallenkamp micromelting point apparatus and are uncorrected. Optical rotations were measured with a Jasco DIP-370 digital polarimeter. Infrared (IR) spectra were recorded in KBr disks with a Shimadzu FTIR-8300 spectrometer. UV spectra were recorded in CH_3OH using a Shimadzu UV-1601 spectrometer. 1D (^1H and ^{13}C) and 2D (DEPT, COSY, HSQC, and HMBC) NMR spectra were recorded on a Bruker DRX-500 instrument (at 500 MHz for ^1H NMR and at 125 MHz for ^{13}C NMR). The chemical shift (δ) values are given in parts per million (ppm) relative to TMS at 0 ppm. The coupling constants (J values) are reported in Hertz (Hz). Standard pulse sequences were used for distortionless enhancement by polarization transfer (DEPT), heteronuclear single quantum coherence (HSQC), and heteronuclear multiple bond coherence (HMBC) experiments. High-resolution electrospray ionization mass spectra (ESI-MS) were obtained with a JEOL HX110A mass spectrometer.

3.2. Substrate, fungus, and the medium

The substrate, 1-aminoanthracene, purchased from Sigma–Aldrich Inc. (Milwaukee, WI, USA) was found to be ca. 90% pure. It was therefore purified by reversed-phase thin-layer chromatography (RP-TLC) by eluting

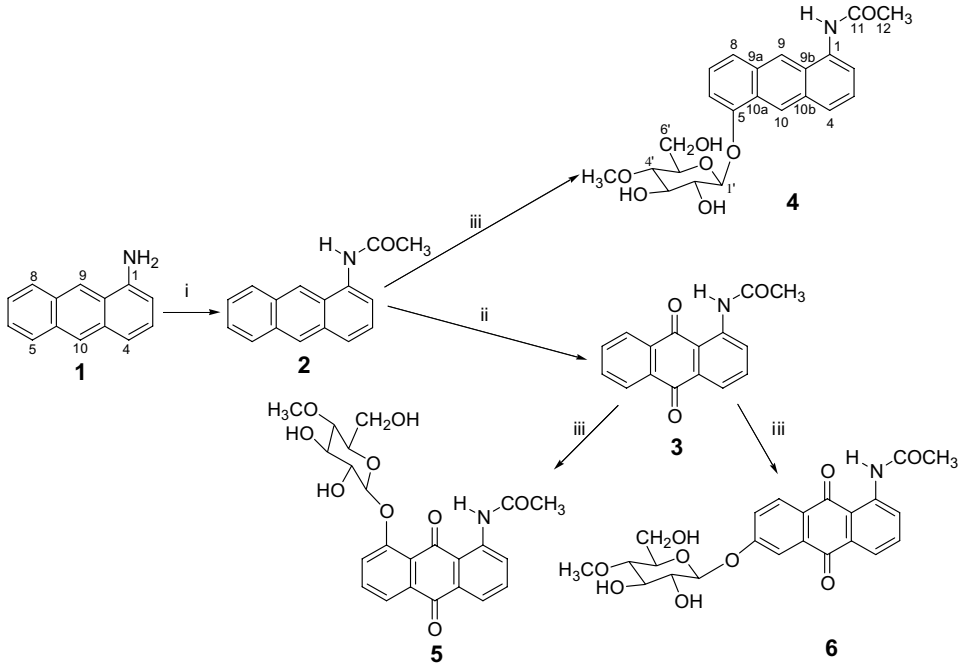

Figure 1. Microbial metabolism of 1-aminoanthracene by *B. bassiana* ATCC 7159. Reagents: (i) Acetylation; (ii) oxidation; (iii) hydroxylation followed by *O*-methylglucosylation.

with MeOH/H_2O (9:1) to obtain material of >99% purity as determined by HPLC analysis. The fungus, *B. bassiana* ATCC 7159 (ATCC, Manassas, USA), was cultured in potato dextrose broth (PDB, Difco, Plymouth, MN, USA) medium.

3.3. Culture and biotransformation procedures

A small scale metabolism experiment of 1-aminoanthracene (**1**) with *B. bassiana* ATCC 7159 was carried out in a 125 mL Erlenmeyer flask containing 50 mL of the PDB medium. The flask was placed in a rotary shaker at 150 rpm and 25 °C. After 3 days, when the fermentation broth turned red, 3.0 mg of 1-aminoanthracene (300 μL of a solution of 10 mg/mL in acetone) was added and the flask was maintained under the same conditions for additional 7 days. Two controls were used. Culture control consisted of fermentation broth of *B. bassiana* ATCC 7159 with the same volume of acetone but without the substrate, and the substrate control consisted of sterile PDB medium with the same amount of a solution of 1-aminoanthracene in acetone but without the microorganism. Both controls were incubated under the same conditions. The preparative scale experiment was carried out in 3× 250 mL flasks, each containing 100 mL of PDB under the same conditions as for the small scale experiment. A total of 40 mg of 1-aminoanthracene were used (13.3 mg/flask).

3.4. Extraction, isolation, and identification of the metabolites

The cultures were filtered to separate mycelia, and the combined culture filtrate (300 mL; pH 2.69) was neutralized with 1.0 N aqueous NaOH and extracted with EtOAc (3× 300 mL). Evaporation of EtOAc under reduced pressure yielded a dark yellow solid (57.5 mg), a portion (53.1 mg) of which was subjected to size-exclusion chromatography on a column of Sephadex LH-20 (3.0 g) and eluted with hexane/acetone (3:2) (30 mL), hexane/acetone (1:1) (30 mL), hexane/acetone (1:2) (30 mL), hexane/acetone (1:3) (30 mL), acetone (20 mL), and finally with MeOH (10 mL). Five fractions (30 mL each) were collected, and evaporation of the solvents yielded fractions A (4.0 mg), B (14.8 mg), C (16.2 mg), D (6.4 mg), and E (4.6 mg). Except for fraction A, all other fractions contained similar spots as judged by both normal phase (hexane/acetone; 1:2) and reversed-phase preparative TLC (CH_3OH/H_2O; 70:30). Fraction A was separated by reversed-phase TLC (MeOH/H_2O; 85:15), yielding 1-acetamidoanthracene (**2**) (0.1 mg) and 1-acetamidoanthraquinone (**3**) (0.3 mg). Fractions B, C, D, and E were separated on normal phase preparative TLC (CH_2Cl_2/MeOH; 87:13) followed by purification on reversed-phase preparative TLC (MeOH/H_2O; 70:30), resulting in the isolation of 1-acetamido-5-[(4′-*O*-methyl-β-D-glucopyranosyl)oxy]anthracene (**4**) (10.6 mg), 1-acetamido-8-[(4′-*O*-methyl-β-D-glucopyranosyl)oxy] anthraquinone (**5**) (8.4 mg) and 1-acetamido-6-[(4′-*O*-methyl-β-D-glucopyranosyl)oxy]anthraquinone (**6**) (8.4 mg).

The freeze–dried mycelia of *B. bassiana* from the above experiment were extracted with 3× 50 mL of MeOH, from which a dark yellow extract (63.1 mg) was obtained after the evaporation of the solvent. This extract was subjected to Sephadex LH-20 (3.0 g) size-exclusion chromatography eluting sequentially with CH_2Cl_2 (30 mL), 5% MeOH in CH_2Cl_2 (30 mL), 10% MeOH in CH_2Cl_2 (20 mL), 20% MeOH in CH_2Cl_2 (10 mL), and MeOH (20 mL). Twenty-three fractions (5 mL each) were collected and combined into eight fractions on the basis of their TLC profiles to provide fractions F (5.2 mg), G (11.3 mg), H (1.8 mg), I (0.5 mg), J (6.0 mg), K (6.6 mg), L (6.8 mg), and M (19.8 mg). Fractions G and H were separated by reversed-phase TLC (MeOH/H_2O, 85:15), leading to the isolation of an additional amount of 1-acetamidoanthracene (**2**) (0.4 mg). Fractions I, J, and K were subjected to normal phase TLC (MeOH/CH_2Cl_2, 13:87) followed by purification on reversed-phase TLC (MeOH/H_2O, 30:70), yielding further quantities of 1-acetamidoanthraquinone (**3**) (7.6 mg), 1-acetamido-8-[(4′-*O*-methyl-β-D-glucopyranosyl)oxy] anthraquinone (**5**) (3.4 mg), and 1-acetamido-6-[(4′-*O*-methyl-β-D-glucopyranosyl)oxy]-anthraquinone (**6**) (3.6 mg).

3.4.1. 1-Acetamido-5-[(4′-*O*-methyl-β-D-glucopyranosyl) oxy]anthracene (4). White powder; mp 250–251 °C; $[\alpha]_D^{25}$ −69.5 (*c* 0.05, MeOH); UV (MeOH) λ_{max} (log ε) 208.0 (5.29), 246.5 (5.47), 357.0 (4.76), 375.0 (4.88), 394.5 (4.78) nm; IR (KBr) ν_{max} 3373, 3246, 3053, 2928, 2889, 1653, 1543, 1462, 1313, 1263, 1215, 1109, 1042, 991 cm^{-1}; HRESIMS *m/z* 428.1712 $[M+1]^+$ (calcd for $C_{23}H_{26}NO_7$, 428.1709). ^1H and ^{13}C NMR data, see Tables 1 and 2.

3.4.2. 1-Acetamido-8-[(4′-*O*-methyl-β-D-glucopyranosyl) oxy]anthraquinone (5). Yellow powder; mp 248 °C (decomposed); $[\alpha]_D^{25}$ −26.5 (*c* 0.03, MeOH); UV λ_{max} (MeOH) (log ε) 223.5 (4.19), 261.5 (4.07) nm, 395.0 (3.51); IR (KBr) ν_{max} 3499, 3275, 2926, 2885, 1699, 1663, 1593, 1531, 1458, 1340, 1288, 1267, 1111, 1094, 1067, 995 cm^{-1}; HRESIMS *m/z* 480.1268 $[M+Na]^+$ (calcd for $C_{23}H_{23}NO_9Na$, 480.1270). ^1H and ^{13}C NMR data, see Tables 1 and 2.

3.4.3. 1-Acetamido-6-[(4′-*O*-methyl-β-D-glucopyranosyl) oxy]anthraquinone (6). Yellow powder; mp 251–252 °C; $[\alpha]_D^{25}$ −100.0 (*c* 0.01, MeOH); UV (MeOH) λ_{max}(log ε) 216.5 (4.45), 268.5 (4.48), 397.5 (3.77) nm; IR (KBr) ν_{max} 3479, 3425, 2966, 2920, 2878, 1705, 1641, 1597, 1522, 1342, 1302, 1273, 1258, 1163, 1082, 1013 cm^{-1}; HRESIMS *m/z* 458.1451 $[M+1]^+$ (calcd for $C_{23}H_{24}NO_9$, 458.1442). ^1H and ^{13}C NMR data, see Tables 1 and 2.

3.5. Acetylation of 1-aminoanthracene

Ac_2O (100 μL) was added with stirring to a solution of 1-aminoanthracene (7.3 mg) in pyridine (300 μL). The mixture was allowed to stand at 25 °C for 1 h, and then dried under nitrogen to afford 1-acetamidoanthracene (**2**) (8.5 mg, 96%).

3.6. Acetylation of 1-aminoanthraquinone

Ac_2O (200 μL) was added with stirring to a solution of 1-aminoanthraquinone (7.3 mg; Sigma–Aldrich, 97%

purity) in pyridine (400 μL). The mixture was allowed to stand at 80 °C for 2 h, and then dried under nitrogen to afford 1-acetamidoanthraquinone (**3**) (8.3 mg, 95%).

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References and notes

1. Chung, K. T.; Cerniglia, C. E. *Mutat. Res.* **1992**, *277*, 201–220.
2. Chung, K. T.; Kirkovsky, L.; Kirkovsky, A.; Purcell, W. P. *Mutat. Res.* **1997**, *387*, 1–16.
3. McCann, J.; Choi, E.; Yamasaki, E.; Ames, B. N. *Proc. Natl. Acad. Sci. U.S.A.* **1975**, *72*, 5135–5139.
4. Fujikawa, K.; Fort, F. L.; Samejima, K.; Sakamoto, Y. *Mutat. Res.* **1993**, *290*, 175–182.
5. Kato, T.; Tadokoro, N.; Tsutsui, M.; Kikugawa, K. *Mutat. Res.* **1991**, *249*, 243–254.
6. Baboshin, M. A.; Baskunov, B. P.; Finkelstein, Z. I.; Golovlev, E. L.; Golovleva, L. A. *Microbiology* **2005**, *74*, 303–309.
7. Moody, J. D.; Freeman, J. P.; Doerge, D. R.; Cerniglia, C. E. *Appl. Environ. Microbiol.* **2001**, *67*, 1476–1483.
8. van Herwijnen, R.; Sprigael, D.; Slot, P.; Govers, H.; Parsons, J. *Appl. Environ. Microbiol.* **2003**, *69*, 186–190.
9. Zhan, J.; Gunatilaka, A. A. L. *J. Nat. Prod.* **2005**, *68*, 1271–1273.
10. Zhan, J.; Gunatilaka, A. A. L. *J. Nat. Prod.* **2006**, *69*, 1525–1527.
11. Zhan, J.; Gunatilaka, A. A. L. *Biocat. Biotrans.* **2006**, *24*, 396–399.
12. Holland, H. L.; Morris, T. A.; Nava, P. J.; Zabic, M. *Tetrahedron* **1999**, *55*, 7441–7460.
13. Haufe, G.; Wölker, D.; Fröhlich, R. *J. Org. Chem.* **2002**, *67*, 3022–3028.
14. Holland, H. L.; Brown, F. M.; Johnson, D. V.; Kerridge, A.; Mayne, B.; Turner, C. D.; van Vliet, A. J. *J. Mol. Catal. B: Enzym.* **2002**, *17*, 249–256.
15. Olivo, H. F.; Peeples, T. L.; Rios, M.; Velazquez, F.; Kim, J.; Narang, S. *J. Mol. Catal. B: Enzym.* **2003**, *21*, 97–105.
16. Pedragosa-Moreau, S.; Archelas, A.; Furstoss, R. *J. Org. Chem.* **1993**, *58*, 5533–5536.
17. Trieff, N. M.; Biagi, G. L.; Ramanujam, V. M. S.; Connor, T. H.; Cantelli-Forti, G.; Guerra, M. C.; Bunce, H., III; Legator, M. S. *Mol. Toxicol.* **1989**, *2*, 53–65.