# Microbial Transformation of Amino- and Hydroxyanthraquinones by *Beauveria bassiana* ATCC 7159

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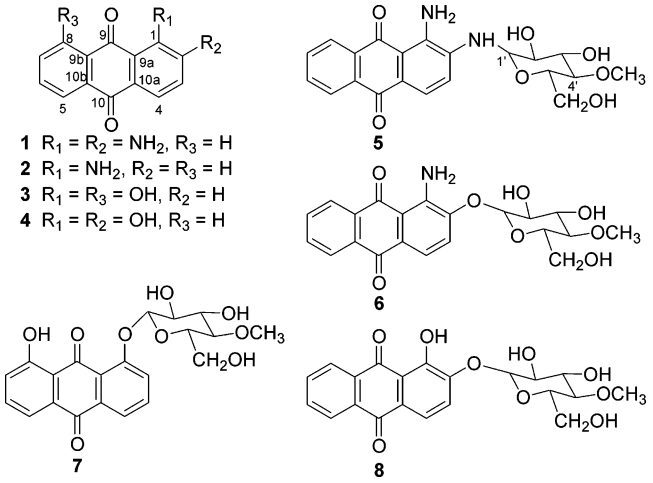
Microbial biotransformation of four amino- and hydroxyanthraquinones catalyzed by *Beauveria bassiana* ATCC 7159 has been studied. Incubation of 1,2-diaminoanthraquinone (**1**) with *B. bassiana* ATCC 7159 afforded 1-amino-2-(4′-*O*-methyl-2*β*-*N*-D-glucopyranosylamino)anthraquinone (**5**) in a hitherto unprecedented biotransformation involving *N*-glycosylation of an amine. Biotransformation of 1-aminoanthraquinone (**2**) yielded 1-amino-2-(4′-*O*-methyl-2*β*-*O*-D-glucopyranosyloxy)anthraquinone (**6**) as a result of microbial hydroxylation of C-2 followed by 4′-*O*-methyl-glucosylation of the newly introduced hydroxyl group. 1,8-Dihydroxyanthraquinone (**3**) and 1,2-dihydroxyanthraquinone (**4**) afforded 8-hydroxy-1-(4′-*O*-methyl-1*β*-*O*-D-glucopyranosyloxy)anthraquinone (**7**) and 1-hydroxy-2-(4′-*O*-methyl-2*β*-*O*-D-glucopyranosyloxy)anthraquinone (**8**), respectively, resulting from 4′-*O*-methyl-glucosylation of the existing hydroxyl groups of the substrates. The efficiency of these conversions suggests that microbial biotransformation reactions offer an attractive alternative to chemical 4′-*O*-methyl-glucosylation of amino- and hydroxyanthraquinones.

Anthraquinones, especially hydroxyanthraquinones, constitute one of the most ubiquitous classes of naturally occurring phenolic compounds, with diverse physiological and pharmacological properties including bacteriostatic,[1a] antifungal,[1b] antibiotic,[1c] anticancer,[1d] antiviral,[1e] and antimalarial[1f] activities. The presence of anthraquinones and their *O*-glycosides is known to contribute to the medicinal properties of some plant-based crude drugs such as aloe[2a] and senna.[2b] In addition to previously known antibacterial and antifungal activities,[3] recent studies have revealed the potential anticancer activity[4] of aminoanthraquinones and their derivatives. Since pharmacological studies of small molecule bioactive compounds are known to be hampered by their weak solubility in water, it was of interest to develop methodologies to prepare water-soluble analogues of hydroxy- and aminoanthraquinones. Herein we wish to report an efficient procedure to prepare *O*- and *N*-glycosides of hydroxy- and aminoanthraquinones utilizing a convenient microbial biotransformation reaction. Although microbial *O*-glycosylation of phenols is well documented,[5] this constitutes the first report of *N*-glycosylation of an aromatic amine by microbial biotransformation. Previous studies on microbial glucosylation of 1- and 2-hydroxyanthraquinones with *Streptomyces aureofaciens* have been reported to result in their corresponding *β*-D-glucosides in poor yields.[6] In a recent study, Zhang et al.[7] screened 21 microbial strains for their ability to glucosylate anthraquinones of rhubarb and found that only *Absidia coerulea* was capable of converting these anthraquinones into their corresponding *O*-glucosides in yields of 6−14%. We and others[8] have previously utilized the microbial strain *Beauveria bassiana* ATCC 7159 for efficient 4′-*O*-methyl-glucosylation of a number of phenolic natural products. When the biotransformation reaction was attempted on four commercially available hydroxy- and aminoanthraquinones employing *B. bassiana* ATCC 7159, in addition to direct 4′-*O*-methyl-glucosylation of the substituents, hydroxylation prior to glycosylation was observed with 1-aminoanthraquinone (**2**). More importantly, this microbial strain was also able to selectively glycosylate only the 2-amino group of 1,2-diaminoanthraquinone (**1**). The incubation of substrates with *B. bassiana* ATCC 7159 and isolation of the products were carried out by the procedures that we have described previously,[8a] and the resulting products were identified on the basis of their spectral data as described below.




HRESIMS of compound **5** isolated in 67% yield from the biotransformation broth of 1,2-diaminoanthraquinone (**1**) suggested a molecular formula of $C_{21}H_{22}N_2O_7$, indicating that a 4′-*O*-methyl-glucose moiety was introduced to the substrate. Analysis of its $^{13}C$ NMR spectrum with the help of DEPT indicated additional signals for five CH, one $CH_2$, and one $OCH_3$ compared with the $^{13}C$ NMR spectrum of **1**, further confirming the presence of a 4′-*O*-methyl-glucose moiety in **5**. The $^{13}C$ NMR chemical shift of the anomeric carbon of **5** at $\delta$ 84.2 suggested that the sugar moiety is not connected to an oxygen atom, but to a nitrogen atom. Therefore, **5** was suspected to be an *N*-4′-*O*-methyl-glucosylation product. In the $^1H$ NMR spectrum, the anomeric proton appeared as a triplet (at $\delta$ 4.50) due to the coupling with neighboring NH and CH protons. These NMR data were consistent with those reported ($\delta_C$ 84.9 and $\delta_H$ 4.43) for related *β*-*N*-glucosides,[9] indicating **5** to be the *β*-*N*-glucosylation product. The presence of a signal due to a chelated $NH_2$ at $\delta$ 8.41 in its $^1H$ NMR spectrum further confirmed that the glucosylation occurred at the C-2 amino group. On the basis of the above evidence and analysis of DQF-COSY, HSQC, and HMBC (Figure 1) spectra, **5** was identified as 1-amino-2-(4′-*O*-methyl-2*β*-*N*-D-glucopyranosylamino)anthraquinone. Incubation of 1-aminoanthraquinone (**2**) with *B. bassiana* ATCC 7159 as for **1** afforded compound **6** in 72% yield. The molecular formula of **6** was deduced as $C_{21}H_{21}NO_8$ from its HRESIMS spectrum. $^1H$ and $^{13}C$ NMR spectra of **6**, in addition to the signals due to 1-ami-

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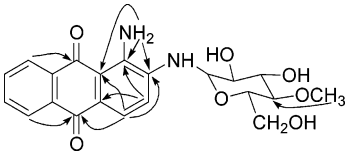


**Figure 1.** Selected HMBC correlations for compound **5**.

noanthraquinone, exhibited signals characteristic of a 4′-*O*-methyl-glucose moiety. The chemical shifts of the anomeric CH in the $^{13}C$ ($\delta$ 100.7) and $^{1}H$ ($\delta$ 4.96, d, $J$ = 7.7 Hz) NMR spectra suggested **6** to be an *O*-4′-*O*-methyl-glucosylation product, indicating that hydroxylation of the substrate occurred prior to the introduction of the 4′-*O*-methyl-glucose moiety. The presence of a set of two protons with *ortho* coupling ($J$ = 8.3 Hz) in the $^{1}H$ NMR spectrum of **6** suggested that the hydroxylation occurred at C-2 or C-4. These two possibilities were differentiated from its HMBC spectrum, which showed a correlation between H-4 ($\delta$ 7.44) and C-10 ($\delta$ 181.7), confirming that the hydroxyl group was introduced at C-2. Compound **6** was therefore identified as 1-amino-2-(4′-*O*-methyl-2$\beta$-*O*-D-glucopyranosyloxy)anthraquinone. Biotransformation of 1,8-dihydroxyanthraquinone (**3**) afforded **7** in 46% yield. HRESIMS analysis suggested a molecular formula of $C_{21}H_{20}O_9$ for **7**, revealing the presence of a 4′-*O*-methyl-glucose moiety, which was further confirmed by $^{13}C$ NMR and DEPT spectra. It was characterized as 8-hydroxy-1-(4′-*O*-methyl-1$\beta$-*O*-D-glucopyranosyloxy)anthraquinone on the basis of its 1D and 2D NMR spectral data. Compound **8** was isolated in 63% yield from the biotransformation broth of 1,2-dihydroxyanthraquinone (**4**). Its molecular formula was deduced as $C_{21}H_{20}O_9$ from HRESIMS, indicating the introduction of a 4′-*O*-methyl-glucose moiety to the substrate. The HMBC correlations of H-1′ ($\delta$ 5.16) and H-4 ($\delta$ 7.67) with C-2 ($\delta$ 151.3) revealed that the sugar moiety is attached to the C-2 hydroxyl group. On the basis of its 1D and 2D NMR data and the evidence presented above, the structure of this product was elucidated as 1-hydroxy-2-(4′-*O*-methyl-2$\beta$-*O*-D-glucopyranosyloxy)anthraquinone (**8**).

Our studies suggest that *B. bassiana* ATCC 7159 is capable of catalyzing three major enzymatic reactions of amino- and hydroxyanthraquinones, namely, hydroxylation, *O*-glycosylation, and *N*-glycosylation. With hydroxyanthraquinones, *O*-glycosylation occurs at both C-1 and C-2 hydroxyl groups with a preference for C-2 glycosylation when both positions contain hydroxyl groups. *O*-Glycosylation of OH at C-1 appears to be less efficient than C-2 probably due to the steric hindrance at the former position. In the case of aminoanthraquinones, it appears that the glycosylation is preferred at C-2, and when this position does not carry any heteroatom amenable for glycosylation, C-2 hydroxylation precedes glycosylation at that position. Furthermore, the efficient conversion of 1,2-diaminoanthraquinone (**1**) to its *N*-glycosylated derivative **5** demonstrates for the first time that *B. bassiana* ATCC 7159 is capable of effecting this biotransformation; the selectivity observed may again be due to the steric hindrance at the C-1 amino group. The method described here provides an efficient approach to prepare *N*-4′-*O*-methyl-glucosyl derivatives of 2-aminoanthraquinones and therefore broadens the application of this microbial catalyst. Two cytochrome P450 inhibitors, bifonazole and clotrimezole,[10] had no noticeable effect at concentrations up to 0.5 mM, suggesting that cytochrome P450 enzymes are not involved in these biotransformation reactions.

## Experimental Section

**General Experimental Procedures.** Melting points were measured on an Electrothermal micromelting point apparatus and are uncorrected. Optical rotations were measured with a Jasco DIP-370 digital polarimeter using MeOH as solvent. IR spectra were recorded on a Shimadzu FTIR-8300 spectrometer in KBr disks and UV spectra in MeOH on a Shimadzu UV-1601 spectrometer. 1D and 2D NMR spectra were recorded in $d_6$-DMSO on a Bruker DRX-500 instrument (500 MHz for $^{1}H$ NMR and 125 MHz for $^{13}C$ NMR). High-resolution ESIMS were obtained with a JEOL HX110A mass spectrometer.

**Biotransformation Procedures.** All biotransformation experiments were carried out in 250 mL Erlenmeyer flasks containing 100 mL of Difco potato dextrose broth (PDB) medium using *B. bassiana* ATCC 7159.[8a] After incubation at 25 °C for 3 days in a rotary shaker at 150 rpm, substrates (**1**−**4**) dissolved in requisite volumes of acetone to make 10 g/L solutions were added and incubated while shaking for an additional 7 days. The amounts of substrates and the number of flasks used for each substrate are as follows: **1** (5.0 mg, 1 flask); **2** (20.0 mg, 4 flasks); **3** (21.5 mg, 4 flasks); **4** (25.0 mg, 5 flasks).

**Extraction and Isolation.** After incubation, the cultures were filtered to separate mycelia from culture media. The filtrates were neutralized and extracted three times with the same volume of EtOAc each time. The mycelia were extracted three times with 50 mL of acetone each time by sonication. For each substrate, the EtOAc and acetone extracts were combined and evaporated to dryness under reduced pressure. The crude products thus obtained (42.7 mg for **1**, 224.9 mg for **2**, 159.8 mg for **3**, and 186.5 mg for **4**) were purified by preparative Si gel TLC using the developing systems MeOH/$CH_2Cl_2$ (13:87), hexane/acetone (2:3), hexane/acetone (1:2), and MeOH/$CH_2Cl_2$/$HCO_2H$ (13:87:1), respectively, to afford **5** (5.8 mg, 67%), **6** (25.1 mg, 72%), **7** (17.1 mg, 46%), and **8** (27.1 mg, 63%).

**1-Amino-2-(4′-*O*-methyl-2$\beta$-*N*-D-glucopyranosylamino)anthraquinone (5):** red powder; mp 197 °C (dec); $[\alpha]_{20}^{D}$ −447.0 (*c* 0.10, MeOH); UV (MeOH) $\lambda_{max}$ (log $\epsilon$) 501 (3.38), 262 (4.10), 208 (4.07) nm; IR (KBr) $\nu_{max}$ 3412, 2930, 1638, 1591, 1560, 1441, 1288, 1109 $cm^{-1}$; $^{1}H$ NMR (500 MHz, $d_6$-DMSO) $\delta$ 8.41 (2H, brs, 1-$NH_2$), 8.21 (1H, dd, $J$ = 7.4, 1.4 Hz, H-8), 8.11 (1H, dd, $J$ = 7.4, 1.4 Hz, H-5), 7.79 (1H, m, H-6), 7.82 (1H, m, H-7), 7.49 (1H, d, $J$ = 8.3 Hz, H-4), 7.35 (1H, d, $J$ = 6.3 Hz, 2-NH), 6.86 (1H, d, $J$ = 8.3 Hz, H-3), 4.50 (1H, t, $J$ = 7.3 Hz, H-1′), 3.62 (1H, brd, $J$ = 11.2 Hz, H-6′), 3.49 (1H, m, H-6′), 3.46 (3H, s, 4′-$OCH_3$), 3.42 (1H, m, H-3′), 3.41 (1H, m, H-2′), 3.33 (1H, m, H-5′), 3.03 (1H, t, $J$ = 9.0 Hz, H-4′); $^{13}C$ NMR (125 MHz, $d_6$-DMSO) $\delta$ 184.2 (C, C-9), 180.6 (C, C-10), 141.3 (C, C-2), 141.2 (C, C-1), 134.6 (C, C-9b), 134.6 (C, C-10b), 133.4 (CH, C-7), 133.1 (CH, C-6), 126.3 (CH, C-8), 125.9 (CH, C-5), 122.6 (C, C-10a), 119.4 (CH, C-4), 112.7 (CH, C-3), 110.7 (C, C-9a), 84.2 (CH, C-1′), 79.2 (CH, C-4′), 77.0 (CH, C-3′), 76.2 (CH, C-5′), 72.4 (CH, C-2′), 60.3 ($CH_2$, C-6′), 59.6 ($CH_3$, $OCH_3$-4′); HRESIMS *m*/*z* 415.1505 $[M + 1]^+$ (calcd for $C_{21}H_{23}N_2O_7$ 415.1505).

**1-Amino-2-(4′-*O*-methyl-2$\beta$-*O*-D-glucopyranosyloxy)anthraquinone (6):** orange powder; mp 245−246 °C (dec); $[\alpha]_{20}^{D}$ −33.1 (*c* 0.01, MeOH); UV (MeOH) $\lambda_{max}$ (log $\epsilon$) 475 (3.86), 310 (3.49), 250 (4.57), 203 (4.44) nm; IR (KBr) $\nu_{max}$ 3427, 3406, 3323, 3240, 2930, 1639, 1545, 1447, 1325, 1290, 1258, 1227, 1099 $cm^{-1}$; $^{1}H$ NMR (500 MHz, $d_6$-DMSO) $\delta$ 8.19 (1H, dd, $J$ = 7.6, 1.3 Hz, H-8), 8.12 (1H, dd, $J$ = 7.6, 1.3 Hz, H-5), 7.87 (1H, dt, $J$ = 7.4, 1.3 Hz, H-7), 7.82 (1H, dt, $J$ = 7.4, 1.3 Hz, H-6), 7.44 (1H, d, $J$ = 8.3 Hz, H-4), 7.31 (1H, d, $J$ = 8.3 Hz, H-3), 4.96 (1H, d, $J$ = 7.7 Hz, H-1′), 3.68 (1H, m, H-6′), 3.52 (1H, m, H-6′), 3.48 (1H, m, H-5′), 3.47 (3H, s, 4′-$OCH_3$), 3.47 (1H, m, H-3′), 3.41 (1H, m, H-2′), 3.09 (1H, t, $J$ = 9.2 Hz, H-4 ); $^{13}C$ NMR (125 MHz, $d_6$-DMSO) $\delta$ 183.9 (C, C-9), 181.7 (C, C-10), 149.7 (C, C-2), 142.7 (C, C-1), 134.0 (CH, C-7), 134.4 (C, C-9b), 133.5 (CH, C-6), 132.8 (C, C-10b), 127.0 (C, C-10a), 126.4 (CH, C-8), 126.2 (CH, C-5), 117.1 (CH, C-3), 116.6 (CH, C-4), 111.9 (C, C-9a), 100.7 (CH, C-1′), 78.9 (CH, C-4′), 75.8 (CH, C-5′), 75.3 (CH, C-3′), 73.2 (CH, C-2′), 60.2 ($CH_2$, C-6′), 59.7 ($CH_3$, $OCH_3$-4′); HRESIMS *m*/*z* 416.1352 $[M + 1]^+$ (calcd for $C_{21}H_{22}NO_8$ 416.1345).

**8-Hydroxy-1-(4′-*O*-methyl-1$\beta$-*O*-D-glucopyranosyloxy)anthraquinone (7):** yellow powder; mp 250−251 °C; $[\alpha]_{25}^{D}$ −65.1 (*c* 0.04, MeOH); UV (MeOH) $\lambda_{max}$ (log $\epsilon$) 407 (2.74), 253 (3.24), 221 (3.40) nm; IR (KBr) $\nu_{max}$ 3395, 2924, 1674, 1647, 1589, 1454, 1288, 1107, 1160, 1026, 995, 961, 833 $cm^{-1}$; $^{1}H$ NMR (500 MHz, $d_6$-DMSO) $\delta$ 7.89 (1H, m, H-2), 7.87 (1H, m, H-3), 7.78 (1H, t, $J$ = 8.0 Hz, H-6), 7.71 (1H, m, H-4), 7.69 (1H, m, H-7), 7.38 (1H, d, $J$ = 8.3 Hz, H-5), 5.21 (1H, d, $J$ = 7.2 Hz, H-1′), 3.66 (1H, m, H-6′), 3.53 (1H, m, H-6′), 3.51 (1H, m, H-5′), 3.49 (3H, s, 4′-$OCH_3$), 3.48 (1H, m, H-2′), 3.47 (1H, m, H-3′), 3.12 (1H, t, $J$ = 9.0 Hz, H-4 ); $^{13}C$ NMR (125 MHz, $d_6$-DMSO) $\delta$ 188.7 (C, C-9), 182.9 (C, C-10), 162.2 (C, C-8), 159.0 (C, C-1), 137.2 (CH, C-6), 136.9 (CH, C-3), 135.7 (C, C-10a), 133.3 (C, C-10b), 125.2 (CH, C-5), 123.1 (CH, C-4), 121.5 (C, C-9a), 121.4 (CH, C-2), 119.2 (CH, C-7), 117.8 (C, C-9b), 101.0 (CH, C-1′), 79.7 (CH, C-4′), 77.2 (CH, C-3′), 76.7 (CH, C-5′), 74.3 (CH, C-2′), 61.0

($CH_2$, C-6′), 60.6 ($CH_3$, $OCH_3$-4′); HRESIMS *m*/*z* 439.0898 $[M + Na]^+$ (calcd for $C_{21}H_{20}O_9Na$ 439.1005).

**1-Hydroxy-2-(4′-*O*-methyl-2-*O*-D-glucopyranosyloxy)anthraquinone (8):** yellow powder; mp 235−236 °C; $[\alpha]_{20}^{D}$ −14.0 (*c* 0.05, MeOH); UV (MeOH) $\lambda_{max}$ (log $\epsilon$) 416 (3.53), 329 (3.23), 247 (4.22), 229 (3.95), 203 (4.10) nm; IR (KBr) $\nu_{max}$ 3572, 3331, 2920, 2853, 1639, 1593, 1462, 1279, 1261, 1097, 1059 $cm^{-1}$; $^1H$ NMR (500 MHz, $d_6$-DMSO) $\delta$ 8.23 (1H, m, H-8), 8.17 (1H, m, H-7), 7.93 (2H, m, H-6 and H-7), 7.67 (1H, d, $J$ = 8.4 Hz, H-4), 7.53 (1H, d, $J$ = 8.4 Hz, H-3), 5.16 (1H, d, $J$ = 7.7 Hz, H-1′), 3.63 (1H, brd, $J$ = 11.4 Hz, H-6′), 3.50 (1H, m, H-6′), 3.47 (1H, m, H-5′), 3.46 (3H, s, 4′-$OCH_3$), 3.45 (1H, m, H-3′), 3.35 (1H, m, H-2′), 3.08 (1H, t, $J$ = 9.3 Hz, H-4′); $^{13}C$ NMR (125 MHz, $d_6$-DMSO) $\delta$ 188.1 (C, C-9), 180.9 (C, C-10), 152.3 (C, C-1), 151.3 (C, C-2), 135.0 (C, C-10b), 134.3 (CH, C-7), 133.3 (CH, C-6), 133.1 (C, C-9b), 126.7 (CH, C-5), 126.6 (CH, C-8), 126.0 (C, C-10a), 120.2 (CH, C-3), 119.6 (CH, C-4), 116.4 (C, C-9a), 99.3 (CH, C-1′), 78.7 (CH, C-4′), 76.4 (CH, C-3′), 75.7 (CH, C-5′), 73.2 (CH, C-2′), 60.0 ($CH_2$, C-6′), 59.7 ($CH_3$, $OCH_3$-4′); HRESIMS *m*/*z* 439.1005 $[M + Na]^+$ (calcd for $C_{21}H_{20}O_9Na$ 439.1005).

**P450 Inhibition Experiments.** The substrates **1**−**4** (5 mg/flask) were incubated with *B. bassiana* ATCC 7159 as for the above biotransformation experiments but in the presence of the P450 inhibitors bifonazole (0.5 mM) and clotrimezole (0.25 mM).[10] TLC analysis of the combined culture and mycelia extracts obtained for each biotransformation reaction following the same workup procedure described above showed that the formation of products **5**−**8** was not affected by these inhibitors.

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