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ANTIBACTERIAL INDOLE ALKALOIDS AND ANTHRAQUINONES FROM A SEWAGE-DERIVED FUNGUS *Eurotium* sp.

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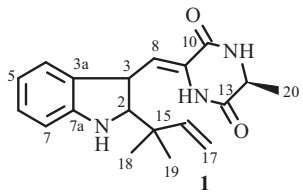
Eurotium, a common fungus closely related to human life, is adapted to high osmotic pressure and can adapt to a low water environment [1]. They are dominant microorganisms that are found during the molding process in the manufacture of Japanese Katsuobushi [2] and Chinese Fuzhuan brick-tea [3]. *Eurotium* sp. are a rich source of bioactive secondary metabolites, such as anthraquinones, benzaldehydes, and diketopiperazines [1]. Some of these metabolites possess antimicrobial [4], antitumor [5], anti-inflammatory [6], and cytotoxic activities [7], as well as antioxidant properties [5], and the ability to degrade pesticide residues [8], among others. Therefore, a comprehensive understanding of the characteristics and secondary metabolites of *Eurotium* would lay a foundation for the reasonable utilization of its resources. Herein, we report the isolation, structural characterization, and biological activities of these compounds.

On the basis of spectral data and by comparison with those reported in the literature, the structures of compounds **1**–**8** were established as neoechinulin A (**1**) [9], L-alanyl-L-tryptophan anhydride (**2**) [10], dihydroxyisoechinulin A (**3**) [11], questin (**4**) [12], questinol (**5**) [13], variecolorquinone A (**6**) [14], asperflavin (**7**) [15], and isotorachrysone-6-*O*-α-D-ribofuranoside (**8**) [16].



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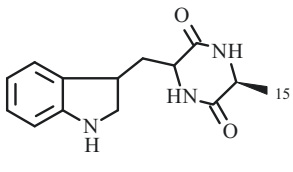

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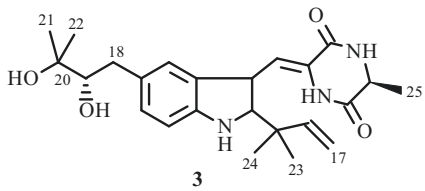

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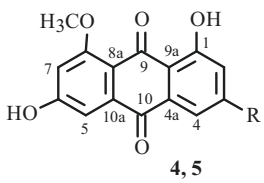
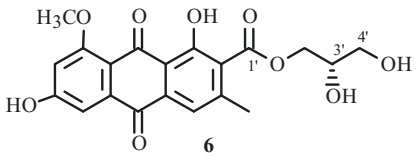


4, 5

4: R = CH_3; **5:** R = CH_2OH



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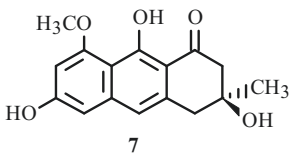

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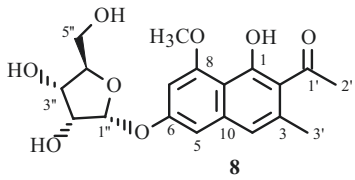

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To the best of our knowledge, compounds **1**–**8** were isolated from *Eurotium* fungus for the first time. In previous reports, many isoechinulin-type alkaloids (**1**–**3**), which usually contain an indole, a diketopiperazine, and a 2-methyl-3-buten-2-yl [17], were isolated from *Aspergillus* species. More than 17 kinds of anthraquinones and their derivatives (**4**–**7**) have been obtained from the fungus of *Aspergillus*. However, naphthyl furanosides were rarely isolated from nature [18]. To date, only five naphthyl furanosides have been reported previously [16].

The fungal strain *Eurotium* sp. M30 XS-2012 was isolated from the sewage produced by a leather factory located in Cangzhou City, Hebei Province, China. Then, it was fermented under liquid medium (mannitol 2.0%, yeast extract 0.3%, flavor essence 1.0%, glucose 1.0%, KH_2PO_4 0.05%, $MgSO_4 \cdot H_2O$ 0.03%, malt sugar 2.0%, corn starch 0.1%, peptone 0.2%, pH 7.0, aged seawater 1000 mL; 500 mL Erlenmeyer flask, 160 L) at 28°C for 14 days on a rotary shaker (120 rpm/min).

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TABLE 1. Antibacterial Activity of Compounds **1–4** (MIC, μM)

Compound	*B. cereus*	*P. vulgaris*	Compound	*B. cereus*	*P. vulgaris*
1	6.25	25.0	**4**	> 25.0	3.13
2	1.56	3.13	Ciprofloxacin	0.78	0.20
3	3.13	> 25.0			

The cultured broth was filtered through cheesecloth to remove fungal mycelia. To remove the larger polar material such as the sugar, the filtrate was passed through macroporous resin and eluted by C_2H_5OH. The solvent was evaporated to obtain a residue (625.0 g) and then extracted with ethyl acetate (EAC). The combined organic layers were evaporated *in vacuo* to afford an extraction residue (49.0 g) that was further separated by silica gel column chromatography (CC) with CH_2Cl_2–MeOH (100:0–1:1) to give seven fractions (Frs. 1–7). Fraction 3 was subjected to Sephadex LH-20 CC (MeOH), and RP-18 silica gel to obtain compound **1** (10.0 mg), compound **2** (8.5 mg), and compound **3** (12.3 mg). Fraction 4 was subjected to silica gel CC ($CHCl_3$–MeOH, 80:1), then separated by Sephadex LH-20 CC ($CHCl_3$–MeOH, 1:1), and further purified by HPLC to afford compounds **4** (10.2 mg), **5** (13.4 mg), and **7** (6.6 mg). Fraction 5 was subjected to ODS CC (MeOH–H_2O, 1:9–0:10). Further purification was carried out using HPLC to give compounds **6** (12.0 mg) and **8** (10.0 mg).

Neoechinulin A (1). $C_{19}H_{21}N_3O_2$. Light yellow powder. ^{1}H NMR (600 MHz, acetone-d_6, δ, ppm, J/Hz): 10.57 (1H, s, NH-1), 7.99 (1H, s, NH-14), 7.67 (1H, s, NH-11), 7.44 (1H, d, J = 8.1, H-4), 7.30 (1H, d, J = 7.9, H-7), 7.11 (1H, m, H-6), 7.10 (1H, s, H-8), 7.06 (1H, t, J = 7.4, H-5), 6.14 (1H, dd, J = 17.4, 10.5, H-16), 5.10 (2H, dd, J = 14.0, 9.5, H-17), 4.29 (1H, q, J = 6.9, H-12), 1.56 (6H, s, H-18, 19), 1.53 (3H, d, J = 7.0, H-20). ^{13}C NMR (150 MHz, acetone-d_6, δ, ppm): 166.2 (s, C-13), 159.6 (s, C-10), 145.1 (d, C-16), 144.0 (s, C-2), 135.2 (s, C-3a), 126.2 (s, C-7a), 125.2 (s, C-3), 121.3 (d, C-6), 119.8 (d, C-5), 118.8 (d, C-7), 111.6 (d, C-17), 111.5 (d, C-8), 110.4 (t, C-4), 103.2 (s, C-9), 51.2 (d, C-12), 39.1 (s, C-15), 27.0 (q, C-18, 19), 19.8 (q, C-20).

L-Alanyl-L-tryptophan Anhydride (2). $C_{14}H_{15}N_3O_2$. White amorphous powder. ^{1}H NMR (600 MHz, DMSO-d_6, δ, ppm, J/Hz): 10.90 (1H, s, NH-1), 8.02 (1H, s, NH-14), 7.91 (1H, s, NH-11), 7.58 (1H, d, J = 7.9, H-4), 7.32 (1H, d, J = 8.0, H-7), 7.05 (1H, d, J = 1.5, H-2), 7.03 (1H, d, J = 8.0, H-6), 6.94 (1H, dd, J = 7.2, 7.0, H-5), 4.12 (1H, s, H-9), 3.60 (1H, m, H-12), 3.25 (1H, dd, J = 14.4, 4.1, H-8), 3.03 (1H, dd, J = 14.5, 4.5, H-8), 0.43 (3H, d, J = 7.0, H-15). ^{13}C NMR (150 MHz, DMSO-d_6, δ, ppm): 168.3 (s, C-13), 167.3 (s, C-10), 136.3 (s, C-7a), 128.3 (s, C-3a), 125.0 (d, C-2), 121.3 (d, C-5), 119.4 (d, C-4), 118.9 (d, C-6), 111.6 (d, C-7), 109.0 (s, C-3), 55.9 (d, C-9), 50.3 (d, C-12), 29.4 (t, C-8), 19.9 (q, C-15).

Dihydroxyisoechinulin A (3). $C_{24}H_{31}N_3O_4$. Colorless oil. ^{1}H NMR (600 MHz, CD_3OD, δ, ppm, J/Hz): 7.37 (1H, d, J = 8.2, H-7), 7.24 (1H, s, H-4), 7.18 (1H, s, H-8), 7.08 (1H, dd, J = 8.3, 1.4, H-6), 6.12 (1H, dd, J = 17.3, 10.6, H-16), 5.12 (2H, ddd, J = 15.7, 11.3, 0.9, H-17), 4.24 (1H, q, J = 7.0, H-12), 3.55 (1H, dd, J = 10.3, 1.9, H-22), 3.08 (1H, dd, J = 14.0, 1.6, H-21), 2.57 (1H, dd, J = 14.0, 10.4, H-21), 1.56 (9H, dd, J = 9.5, 5.9, H-18, 19, 20), 1.26 (3H, s, H-24), 1.24 (3H, s, H-25). ^{13}C NMR (150 MHz, CD_3OD, δ, ppm): 167.4 (s, C-13), 160.9 (s, C-10), 144.9 (d, C-16), 144.8 (s, C-2), 134.1 (s, C-7a), 131.9 (s, C-5), 126.0 (s, C-3a), 123.1 (d, C-6), 122.9 (s, C-9), 118.9 (d, C-4), 113.2 (t, C-17), 111.1 (d, C-7), 110.9 (d, C-8), 102.7 (s, C-3), 80.2 (d, C-22), 72.4 (s, C-23), 51.3 (d, C-12), 39.1 (s, C-15), 37.8 (t, C-21), 26.8 (q, C-18), 26.7 (q, C-19), 24.7 (q, C-24), 23.6 (q, C-25), 19.5 (q, C-20).

Questin (4). $C_{16}H_{12}O_5$. Yellow needles, mp 297–299°C (MeOH) [12]. ^{1}H NMR (600 MHz, DMSO-d_6, δ, ppm, J/Hz): 13.24 (1H, s, 1-OH), 11.25 (1H, s, 6-OH), 7.43 (1H, d, J = 0.9, H-4), 7.22 (1H, d, J = 2.4, H-5), 7.13 (1H, d, J = 0.6, H-2), 6.86 (1H, d, J = 2.3, H-7), 3.91 (3H, s, 8-OCH_3), 2.40 (3H, s, H-11). ^{13}C NMR (150 MHz, DMSO-d_6, δ, ppm): 186.8 (s, C-9), 182.8 (s, C-10), 164.9 (s, C-6), 163.9 (s, C-8), 162.2 (s, C-1), 147.1 (s, C-3), 137.3 (s, C-10a), 132.5 (s, C-4a), 124.6 (d, C-2), 119.6 (d, C-4), 114.9 (s, C-9a), 113.2 (s, C-8a), 107.4 (d, C-5), 105.5 (d, C-7), 56.8 (q, 8-OCH_3), 21.8 (q, C-11).

Questinol (5). $C_{16}H_{12}O_6$. Light yellow amorphous powder. ^{1}H NMR (600 MHz, DMSO-d_6, δ, ppm, J/Hz): 13.26 (1H, s, 1-OH), 11.24 (1H, s, 6-OH), 7.55 (1H, d, J = 1.4, H-4), 7.18 (2H, d, J = 2.3, H-2, 5), 6.81 (1H, d, J = 2.3, H-7), 4.57 (2H, s, H-11), 3.89 (3H, s, 8-OCH_3). ^{13}C NMR (150 MHz, DMSO-d_6, δ, ppm): 186.8 (s, C-9), 182.7 (s, C-10), 164.9 (s, C-6), 163.9 (s, C-8), 162.2 (s, C-1), 151.7 (s, C-3), 137.3 (s, C-10a), 132.5 (s, C-4a), 121.4 (d, C-2), 116.3 (d, C-4), 115.5 (s, C-9a), 113.1 (s, C-8a), 107.4 (d, C-5), 105.4 (d, C-7), 62.5 (t, C-11), 56.8 (q, 8-OCH_3).

Variecolorquinone A (6). $C_{20}H_{18}O_9$. Yellow amorphous powder. ^{1}H NMR (600 MHz, DMSO-d_6, δ, ppm, J/Hz): 13.6 (1H, s, 1-OH), 11.3 (1H, s, 6-OH), 7.46 (1H, s, H-4), 7.17 (1H, d, J = 2.3, H-5), 6.81 (1H, d, J = 2.2, H-7), 4.38 (1H, dd, J = 11.1, 4.0, H-2′), 4.22 (1H, dd, J = 11.1, 6.5, H-2′), 3.90 (3H, s, 8-OCH_3), 3.78 (1H, m, H-3′), 3.42 (2H, m, H-4′), 2.37 (3H, s, CH_3-3). ^{13}C NMR (150 MHz, DMSO-d_6, δ, ppm): 186.5 (s, C-9), 182.2 (s, C-10), 166.4 (s, C-6), 165.2 (s, C-8), 164.0 (s,

C-1′), 158.9 (s, C-1), 143.3 (s, C-3), 137.0 (s, C-10a), 132.6 (s, C-4a), 129.4 (s, C-2), 119.6 (d, C-4), 115.1 (s, C-9a), 112.9 (s, C-8a), 107.6 (d, C-5), 105.4 (d, C-7), 69.7 (d, C-3′), 67.4 (t, C-2′), 63.1 (t, C-4′), 56.8 (q, 8-OCH_3), 19.9 (q, CH_3-3).

Asperflavin (7). $C_{16}H_{16}O_5$. Greenish amorphous powder. ^{1}H NMR (600 MHz, CD_3OD, δ, ppm, J/Hz): 15.17 (1H, s, 9-OH), 6.79 (1H, s, H-10), 6.54 (1H, d, J = 1.8, H-5), 6.45 (1H, d, J = 1.8, H-7), 3.93 (3H, s, 8-OCH_3), 3.01 (2H, q, J = 15.8, H-4), 2.83, 2.74 (each 1H, d, J = 17.1, H-2), 1.37 (3H, s, H-11). ^{13}C NMR (150 MHz, CD_3OD, δ, ppm): 202.7 (s, C-1), 165.1 (s, C-9), 161.5 (s, C-8), 160.6 (s, C-6), 142.3 (s, C-10a), 137.0 (s, C-4a), 116.3 (d, C-10), 108.9 (s, C-8a), 108.8 (s, C-9a), 102.1 (d, C-5), 97.5 (d, C-7), 69.9 (s, C-3), 54.9 (q, 8-OCH_3), 51.1 (t, C-2), 42.7 (t, C-4), 27.5 (q, C-11).

Isotorachrysone-6-*O*-α-D-ribofuranoside (8). $C_{19}H_{22}O_8$. Yellow needles, mp 174–176°C (MeOH) [15]. ^{1}H NMR (600 MHz, CD_3OD, δ, ppm, J/Hz): 7.01 (2H, m, H-4, 5), 6.79 (1H, d, J = 1.7, H-7), 5.78 (1H, d, J = 4.4, H-1″), 4.26 (1H, dd, J = 6.2, 4.6, H-2″), 4.19 (1H, m, H-4″), 4.15 (1H, dd, J = 6.4, 3.0, H-3″), 3.75 (1H, dd, J = 12.1, 3.4, H-5″), 3.69 (1H, dd, J = 12.1, 3.9, H-5″), 2.61 (3H, s, H-2′), 2.30 (3H, s, H-3′). ^{13}C NMR (150 MHz, CD_3OD, δ, ppm): 206.5 (s, C-1′), 157.7 (s, C-8), 156.8 (s, C-6), 152.9 (s, C-1), 137.6 (s, C-10), 134.2 (s, C-3), 122.4 (s, C-2), 118.9 (d, C-4), 109.0 (s, C-9), 103.2 (d, C-5), 100.6 (d, C-1″), 98.5 (d, C-7), 86.5 (d, C-4″), 72.1 (d, C-2″), 69.8 (d, C-3″), 61.8 (d, C-5″), 55.6 (q, C-4′), 31.1 (q, C-2′), 18.8 (q, C-3′).

The antimicrobial activity of compounds **1**–**8** against Gram-positive bacteria (*B. cereus*) and Gram-negative bacteria (*P. vulgaris*) were determined by a serial dilution technique using 96-well microtiter plates [19]. Ciprofloxacin was used as the positive control. DMSO (25 μM) was used as the negative control. Only compounds **1**–**4** showed obvious antibacterial activity (Table 1). The other isolated compounds were inactive with MICs > 25 μM.

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