It was not until Alexander Fleming discovered penicillin G from Penicillium notatum in 1928 that fungal microorganisms suddenly became a hunting ground for novel drug leads.1,2 Hence, many pharmaceutical companies and research groups were motivated to start sampling and screening large collections of fungal strains for antibiotic,3,4 antifungal,5 antiviral,6 antitubercular,7 antineoplastic,8 and other pharmacologically active agents.9,10 The genus Aspergillus, which contains approximately 180 recognized species, has been proven a logically active agents.12) The genus Aspergillus oryzae, which conventionally named as speradine F, was obtained as yellow crystal and was analyzed to have the molecular formula C_{21}H_{22}N_{2}O_{7} through positive high-resolution electrospray ionization mass spectroscopy (HR-ESI-MS) (m/z: 415.1506 [M + H]^+). Its NMR data (Tables 1, 2), combined with distortionless enhancement by polarization transfer (DEPT) and heteronuclear multiple quantum coherence (HMOC) spectrum analyses, revealed twenty-one carbon signals, including three methyls, one methylene, five methines, and eleven quaternary carbons. The planer structure of 1 was revealed through correlation spectroscopy (COSY) and heteronuclear multiple bond connectivity (HMBC) spectrum analyses (Fig. 1). The COSY correlations of H-8 with H-4 and H-9 as well as H-11 with H-10 and H-12 demonstrated the connections from H-4 to H-9 via H-8 and from H-10 to H-12 via H-11. The HMBC correlations of H-4 with C-3, H-9 with C-3a and C-9a, H-10 with C-3a and C-9, H-11 with C-9a, and H-12 with C-12a connected rings A and B together. The HMBC correlations of H-4 with C-5 and C-7, H-8 with C-14, and H-14 with C-7 and C-15 linked rings C to B. The HMBC correlations from H-13 to C-2 and C-12a, and from H-4 to C-2 linked ring D to rings A and B. The HMBC correlations from H-4 to C-3, C-5, and C-20 linked ring E to rings B and C. The HMBC correlations of H-15 with C-16 (4-bond correlation, weak) and 17-OH with C-16, C-17, and C-20 linked ring F to rings C and E. The HMBC correlations of H-19 with C-17 and C-18 linked the last acetyl group to ring F. Furthermore, nuclear Overhauser effect spectroscopy (NOESY) data and X-ray diffraction results help in determine the relative configuration of 1, as shown in Figs. 1, 3, and 5.

Compound 2, which is trivially named as speradine G, was obtained as orange oil and was analyzed to have the molecular formula C_{16}H_{18}N_{2}O_{3} through positive HR-ESI-MS (m/z: 287.1388 [M + H]^+). Calcd for C_{16}H_{18}N_{2}O_{3}: 287.1390). Its NMR data (Tables 1, 2), combined with DEPT and HMQC spectrum analyses, revealed sixteen carbon signals, including three methyls, one methylene, five methines, and seven quaternary carbons. The one dimensional (1D)-NMR data of 2 indicated that its structure is similar to that of 1, except for the disappearance of five carbons from C-16 to C-20, two obviously up-field shifts of C-3 (from δ_{c} 83.0 s to δ_{c} 70.5 s) and C-7 (from δ_{c} 69.1 s to δ_{c} 57.0 s), and an obviously downfield shift of C-5 (from δ_{c} 103.3 s to δ_{c} 174.1 s). The similar COSY, HMBC and NOESY correlations of 2 and 1 from rings A to D, and two left hydroxy or amidogen protons of 2 (δ_{c} 7.48 brs and 5.38 brs) suggested 2 was 3,6-hydrolytic and 5-oxidative degradation derivative of 1 (Fig. 1).

Compound 3, which is trivially named as speradine H, was obtained as orange powder and was analyzed to have the molecular formula C_{20}H_{18}N_{2}O_{4} through positive HR-ESI-MS (m/z: 373.1162 [M + Na]^+). Calcd for C_{20}H_{18}N_{2}NaO_{4}: 373.1159). Its NMR data (Tables 1, 2), combined with DEPT and HMQC spectrum analyses, revealed twenty carbon signals, including four methyls, one methylene, four methines, and eleven quaternary carbons. The 1D-NMR data of 3 indicated that its...
structure is similar to that of 1, except for the disappearance of C-20 ($\delta_{C} 107.0$ s), an obviously upfield shift of C-17 (from $\delta_{C} 88.0$ s to $\delta_{C} 54.2$ t), and five obviously downfield shifts of C-3 (from $\delta_{C} 83.0$ s to $\delta_{C} 125.7$ s), C-4 (from $\delta_{C} 53.6$ d to $\delta_{C} 125.7$ s), C-5 (from $\delta_{C} 103.3$ s to $\delta_{C} 165.1$ s), C-8 (from $\delta_{C} 54.7$ d to $\delta_{C} 152.8$ s), and C-9 (from $\delta_{C} 27.7$ t to $\delta_{C} 123.3$ d). The similar COSY and HMBC correlations of 3 and 1 suggested rings A, D and the four-carbon chain from C-16 to C-19 were reserved. In view of the five obviously downfield shifts in rings B and C, the HMBC correlations of H-9 with C-3a, C-4, and C-5 (4-bond correlation, weak), H-12 with C-3 (4-bond correlation, weak), as well as H-14 with C-7 and C-8 suggested ring B was dehydrated and dehydrogenized, and C-5 of ring C was oxidated, which were consistent to its molecular formula and degree of unsaturation. So the structure of 3 was deduced as shown in Fig. 1.

To explain the biogenetic origin of speradines F–H (1–3), a plausible biosynthetic pathway is proposed in Fig. 4, which is very similar to the reported biosynthesis of CPA.17,18)
The compounds 1–3 were tested for their cytotoxic effects on the Hela cell line using the Sulforhodamine B (SRB) method and on the HL-60 and K562 cell lines using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) method.19) Unfortunately, the results showed that all of their IC50 values were larger than 30 $\mu$g/mL.

### Experimental

**General Experimental Procedures** Optical rotations were obtained from an Anton Paar MCP-200 digital polarimeter. IR spectra were recorded on a Nicolet Avatar 670 spectrophotometer. 1H-NMR, 13C-NMR, DEPT spectra and 2D-NMR were recorded on a Bruker Biospin Avance.

| Position | 1 | 2 | 3 |
|----------|---|---|---|
| 4 | 3.15 (1H, d, 8.3) | 3.19 (1H, d, 9.4) | 3.03 (1H, d, 8.3) |
| 8 | 2.40 (1H, m) | 2.27 (1H, ddd, 12.8, 9.4, 5.0) | 2.31 (1H, d, 9.4) |
| 9-1 | 2.68 (1H, dd, 13.6, 5.3) | 2.98 (1H, ddd, 13.3, 12.8) | 2.90 (1H, d, 13.3) |
| 9-2 | 2.49 (1H, ddd, 13.6, 12.6) | 2.66 (1H, ddd, 13.3, 5.0) | 2.64 (1H, d, 5.0) |
| 10 | 6.89 (1H, d, 7.7) | 6.88 (1H, d, 6.7) | 6.89 (1H, d, 6.0) |
| 11 | 7.30 (1H, ddd, 7.8, 7.7) | 7.26 (1H, ddd, 7.8, 7.7) | 7.27 (1H, ddd, 7.8, 7.7) |
| 12 | 6.69 (1H, d, 7.8) | 6.67 (1H, d, 7.8) | 6.69 (1H, d, 7.8) |
| 13 | 3.17 (3H, s) | 3.17 (3H, s) | 3.17 (3H, s) |
| 14 | 1.75 (3H, s) | 1.40 (3H, s) | 1.92 (3H, s) |
| 15 | 1.56 (3H, s) | 1.32 (3H, s) | 1.92 (3H, s) |
| 17 | 2.41 (3H, s) | 2.45 (2H, s) | 2.37 (3H, s) |
| 17-OH | 6.41 (1H, brs) | 7.48 (1H, brs) | 5.38 (1H, brs) |
| 3-OH or 6-NH | 5.41 (1H, brs) | 5.41 (1H, brs) | 5.41 (1H, brs) |
| 5-OH or 20-OH | 4.68 (1H, brs) | 4.68 (1H, brs) | 4.68 (1H, brs) |
Fig. 2. Key COSY and HMBC Correlations of Compounds 1–3

Fig. 3. NOESY Correlations of Compounds 1 and 2

Fig. 4. Plausible Biosynthetic Pathway of Compounds 1–3
III spectrometer using tetramethylsilane (TMS) as the internal standard. HRESIMS were obtained by an Agilent Q-TOF 6520 LC mass spectrometer. Semipreparative HPLC was performed using an ODS column (ODS-A, 10×250mm, 5μm) at 5 mL/min.

Fungal Material The fungus *A. oryzae* was isolated from marine sediments collected from Langqi Island, Fujian, China. It was identified according to its morphological characteristics and ITS by Beijing Sunbiotech Co., Ltd., and preserved in our laboratory at −80°C. The producing strain was prepared on Martin medium and stored at 4°C.

Fermentation and Extraction The fungus *A. oryzae* was cultured under static conditions at 28°C for 34 d in 1000-mL conical flasks containing the liquid medium (400 mL/flask), composed of glucose (10 g/L), maltose (20 g/L), mannitol (20 g/L), monosodium glutamate (10 g/L), KH₂PO₄ (0.5 g/L), MgSO₄·7H₂O (0.3 g/L), yeast extract (3 g/L), and seawater. The fermented whole broth (40 L) was filtered through cheese cloth to separate supernatant from mycelia. The former was extracted two times with EtOAc to give an EtOAc solution, while the latter was extracted three times with acetone. The acetone solution was concentrated under reduced pressure to give a crude extract (42.3 g).

Purification The crude extract of the fungus *A. oryzae* was separated into five fractions on a Si gel column using a step gradient elution of petroleum ether, CHCl₃. IR (KBr) cm⁻¹: 3428, 2925, 1699, 1614, 1475, 1373, 1299. ¹H- and ¹³C-NMR data (see Tables 1, 2). HR-ESI-MS m/z: 415.1506 [M+H]+ (Caled for C₂₅H₂₂NaO₄: 415.1500). Speradine G (2): Orange oil (CHCl₃). [α]D²⁰ = −25.6 (c=0.13, CHCl₃). IR (KBr) cm⁻¹: 3273, 2929, 1724, 1679, 1614, 1475, 1368. ¹H- and ¹³C-NMR data (see Tables 1, 2). HR-ESI-MS m/z: 287.1388 [M+H]+ (Caled for C₁₆H₁₉N₂O₃: 287.1390).

Speradine H (3): Orange powder (CHCl₃). IR (KBr) cm⁻¹: 3436, 2921, 1736, 1704, 1634, 1458, 1319, 1287. ¹H- and ¹³C-NMR data (see Tables 1, 2). HR-ESI-MS m/z: 373.1162 [M+Na]+ (Caled for C₂₀H₂₀Na₂O₄: 373.1159).

Biological Assays The cytotoxic activity for the HL-60 and K562 cancer cell lines was evaluated by the MTT method and the Hela cancer cell line was evaluated by the SRB method. Doxorubicin was used as the reference drug.

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