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^{1}H NMR REASSIGNMENT FOR *Z*/*E*-BENZOMALVINS B AND ABSOLUTE CONFIGURATION OF BENZOMALVIN C

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*Benzomalvins B, C, and E (**1**, **2**, and **3**) were simultaneously obtained from the marine fungus* Aspergillus *sp. isolated from the soft coral* Sinularia *sp., collected from South China Sea. Their structures were elucidated on the basis of NMR spectroscopic data and by comprehensive comparison with those previously reported in the literature. The stability of the double bond of* E*-benzomalvin B (**1**) under different light conditions was investigated, and **1** was transformed to* Z*-benzomalvin B (**1′**) with UV 365 nm irradiation. In particular, the incorrect ^{1}H NMR data of **1′** in the literature was reassigned for the first time. Moreover, the absolute configuration of benzomalvin C (**2**) was also reported for the first time by X-ray single crystal diffraction.*

Keywords: *Aspergillus* sp., benzomalvins, *E–Z* isomerisation, X-ray single crystal diffraction.

Coral-derived microorganisms are known for their inherent ability to produce novel products of pharmaceutical importance. Nearly 370 marine natural products (MNPs) have been isolated from coral-derived microorganisms [1, 2]. Benzodiazepines are a type of interesting secondary metabolites derived from phenylalanine and anthranilic acid [3]. The majority of naturally occurring benzodiazepines is reported from filamentous fungi and actinomycetes of the genera *Penicillium*, *Aspergillus*, and *Streptomyces* [4]. Some of them showed inhibitory activity against substance P at the neurokinin NK1 receptor in the guinea pig, rat, and human [5], DPPH radical-scavenging activity [6], and antitumor activity [7].

As part of our ongoing investigation on new bioactive natural products from marine fungi in the South China Sea [8–10], three benzodiazepine-quinazolinones (**1–3**) were obtained from the fungus *Aspergillus* sp. by column chromatography and semipreparative HPLC. Their structures were identified as *E*-benzomalvin B (**1**) [11] and benzomalvins C (**2**) [3], E (**3**) [12] by spectroscopic methods, including ^{1}H and ^{13}C NMR. Herein, *E*-benzomalvin B (**1**) was transformed to *Z*-benzomalvin B (**1′**) with UV 365 nm irradiation. In particular, the incorrect ^{1}H NMR data of *Z*-benzomalvin B (**1′**) in the literature [11] was reassigned for the first time. Importantly, the absolute configuration of benzomalvin C (**2**) was also reported for the first time by X-ray single crystal diffraction.

E-benzomalvin B (**1**) was isolated as colorless crystals. The molecular formula $C_{24}H_{17}N_3O_2$ (18 degrees of unsaturation) was determined by EI-MS analysis together with NMR data. The numbers of hydrogen and carbon atoms observed in the ^{1}H and ^{13}C NMR spectra were in agreement with the molecular formula. One methyl carbon signal (δ_C 36.1), nine sp^2 quaternary carbon signals (δ 165.3, 161.2, 151.6, 147.1, 132.8, 133.5, 129.8, 129.6, 122.3) and 12 sp^2 protonated carbon signals (δ 135.5, 132.9, 132.1, 131.3, 129.7, 129.4, 129.3, 129.1, 128.7, 128.4, 128.1, 127.4) were observed in the ^{13}C NMR spectrum. In the ^{1}H NMR spectrum, 11 hydrogen signals were observed. The double bond hydrogen signal was at δ 7.05, and the methyl hydrogen signal at δ 3.34. The proton signals (δ 8.25, 7.86, 7.84, 7.77, 7.65, 7.64, 7.61, 7.54, 7.25) were attributed to aromatic protons. By comparison with data previously reported in the literature, the structure of **1** was determined as *E*-benzomalvin B [11].

The effects of the double bond of **1** under different light conditions (UV 365 nm, 395 nm, 410 nm, and blue light) and solvents (DMSO, acetone, methanol, and acetonitrile) were investigated. Interestingly, a double-bond isomerization was observed in which *E*-benzomalvin B (**1**) in the above solutions slowly rearranged to give *Z*-benzomalvin B (**1′**) with UV 365 nm irradiation at room temperature.

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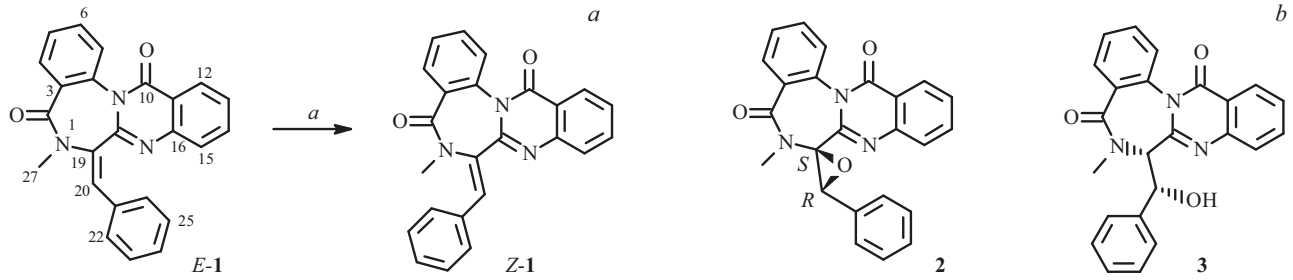

Fig. 1. The double bond isomerization of compound **1** (*a*); the structure of compounds **2** and **3** (*b*).

Z-Benzomalvin B had been previously synthesized from (–)-benzomalvin A by NBS, AIBN, and DBU [11]. However, several differences in ^{1}H NMR chemical shift assignments between our data and the literature were noted. Closer examination revealed that the original ^{1}H NMR data had been incorrectly reported. The correct NMR data for *Z*-benzomalvin B is reported in this report.

Benzomalvin C (**2**) was isolated as colorless crystals. The molecular formula $C_{24}H_{17}N_3O_3$ (18 degrees of unsaturation) was determined by EI-MS analysis together with NMR data. Compound **2** showed similar ^{1}H and ^{13}C NMR data to **1**. The NMR difference between **2** and **1** was a methine signal (δ_H 3.89, δ_C 68.1) in **2** instead of a double bond proton signal (δ_H 7.05, δ_C 132.9) in **1**. Finally, the structure of **2** was determined as benzomalvin C [3]. Benzomalvin C was first isolated from the *Penicillium* sp. in 1994 [3], then reported from the fungus *Penicillium* sp. FN070315 [12] and the interrhizospheric fungus *Penicillium* sp. SYPF 8411 [13]. It should be mentioned that only the planar structure and relative configuration of benzomalvin C were reported, and its absolute configuration has not been determined until now.

To elucidate the structure unambiguously, we undertook a single X-ray diffraction study. Ultimately, by slow crystallization in MeOH, tiny single crystals of **2** formed, which were suitable for X-ray diffraction analysis. As a result, the planar and stereo structure of **2** were firmly established with the absolute configuration of 19*S*, 20*R* through refinement of Flack′s parameter [x = 0.05(9)]. To the best of our knowledge, this is the first time that the absolute configuration of benzomalvin C was identified.

EXPERIMENTAL

Fungus Material, Fermentation, Extraction, and Isolation. The fungal strain was isolated from the soft coral *Sinularia* sp., which was collected from South China Sea in 2015. The strain was identified as *Aspergillus* sp., according to morphological traits and molecular identification. The sequence data derived from the fungal strain has been submitted and deposited in GenBank with the accession No. KY235298. A voucher specimen was deposited at the School of Medicine and Pharmacy, Ocean University of China, Qingdao, P. R. China. The strain was cultured in 500 mL Erlenmeyer flask containing 60 g rice and 60 mL pure water (total 50 flasks). Cultivation at room temperature for 30 days and extraction three times with EtOAc (200 mL per flask) gave an organic extract (9.3 g). The organic extract was separated and purified by a combination of silica gel column chromatography, Sephadex LH-20, and semi-preparative HPLC to yield benzomalvin B (**1**) (210.5 mg), benzomalvin C (**2**) (65.5 mg), and benzomalvin E (**3**) (131.5 mg).

Isomerization of *E*-Benzomalvin B (1). Solutions of **1** (1 mg/mL) in solvents DMSO, acetone, methanol, and acetonitrile were irradiated under different light wavelengths (UV 365 nm, 395 nm, 410 nm, and blue light) at room temperature for 30 min. The reaction mixture was monitored and purified by HPLC to afford **1′**.

***E*-Benzomalvin B (1)**. White crystals. ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm, J/Hz): 8.25 (1H, dd, J = 7.6, 1.2, H-12), 7.86 (1H, m, H-4), 7.84 (1H, m, H-14), 7.77 (1H, dd, J = 7.6, 1.2, H-15), 7.65 (1H, m, H-7), 7.64 (1H, m, H-13), 7.61 (1H, m, H-5), 7.54 (1H, td, J = 7.6, 1.2, H-6), 7.25 (5H, overlapped, H-22–26), 7.05 (1H, s, H-20), 3.34 (3H, s, H-27). ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 165.3 (C-2), 161.2 (C-10), 151.6 (C-18), 147.1 (C-16), 135.5 (C-14), 133.5 (C-3), 132.9 (C-20), 132.8 (C-21), 132.1 (C-5), 131.3 (C-4), 129.8 (C-8), 129.7 (C-24), 129.6 (C-19), 129.4 (C-23, 25), 129.3 (C-6), 129.1 (C-22, 26), 128.7 (C-13), 128.4 (C-15), 128.1 (C-12), 127.4 (C-7), 122.3 (C-11), 36.1 (C-27). (+)-ESI-MS *m/z* 380 $[M + H]^+$.

***Z*-Benzomalvin B (1′)**. White crystals. ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm, J/Hz): 8.38 (1H, dd, J = 8.0, 1.3, H-12), 7.99 (1H, dd, J = 8.0, 1.3, H-4), 7.85 (1H, dd, J = 8.0, 1.3, H-14), 7.83 (1H, m, H-7), 7.59 (1H, m, H-15), 7.56 (1H, m, H-13), 7.55 (1H, m, H-5), 7.49 (1H, m, H-6), 7.37 (5H, overlapped, H-22–26), 6.91 (1H, s, H-20), 3.21 (3H, s, H-27). (+)-ESI-MS *m/z* 380 $[M + H]^+$.

Benzomalvin C (2). White crystals. ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm, J/Hz): 8.25 (1H, dd, J = 8.0, 1.2, H-12), 7.96 (1H, dd, J = 8.0, 1.2, H-4), 7.93 (1H, br.d, J = 8.0, H-15), 7.84 (1H, m, H-14), 7.58 (1H, m, H-5), 7.55 (1H, m, H-13), 7.43 (1H, td, J = 8.0, 1.2, H-6), 7.18 (1H, m, H-24), 7.07 (2H, t, J = 7.5, H-23, 25), 6.89 (1H, br.d, J = 8.0, H-7), 6.62 (2H, br.d, J = 7.5, H-22, 26), 3.89 (1H, s, H-20), 3.25 (3H, s, H-27). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 165.8 (C-2), 160.5 (C-10), 148.1 (C-18), 146.1 (C-16), 135.4 (C-14), 132.1 (C-8), 131.7 (C-3), 131.1 (C-6), 130.3 (C-21), 129.8 (C-4), 129.5 (C-24), 129.2 (C-5), 128.7 (C-7), 128.4 (C-23, 25), 128.4 (C-13), 128.3 (C-15), 127.7 (C-12), 126.0 (C-22, 26), 122.0 (C-11), 72.3 (C-19), 68.1 (C-20), 29.1 (C-27). (+)-ESI-MS *m/z* 396 $[M + H]^+$.

X-Ray Crystallographic Analysis of 2. White crystals of **2** were obtained from MeOH. The crystal data were recorded at 293 K on an Agilent Gemini Ultra diffractometer with Cu Kα radiation (λ = 1.54718 Å). The structure was solved by direct methods (SHELXL-97) and refined using full-matrix least-squares difference Fourier techniques. All nonhydrogen atoms were refined anisotropically, and all hydrogen atoms were placed in idealized positions and refined as riding atoms with the relative isotropic parameters.

Crystal Data for 2. $C_{24}H_{17}N_3O_3$, M_r = 395.41, monoclinic, space group $P2_1$ with a = 11.2116 (5) Å , b = 23.4715 (8) Å, c = 23.2885 (8) Å, β = 91.595(4), V = 6126.0 (4) Å^3 , Z = 2, D_x = 1.286 g/cm^3, μ(Cu Kα) = 0.705 mm^{-1}, and F(000) = 2472. Crystal dimensions: 0.08 × 0.07 × 0.07 mm^3. Independent reflections: 20129. The final R_1 values were 0.1232, Flack parameter = 0.05(9), and wR2 = 0.3142 ($I > 2\sigma$ (I)). Crystallographic data have been deposited in the Cambridge Crystallographic Data Centre No. 1982703.

Benzomalvin E (3). White crystals. ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm, J/Hz): 8.24 (1H, dd, J = 8.0, 1.5, H-12), 7.93 (1H, ddd, J = 8.0, 7.0, 1.5, H-4), 7.88 (1H, dd, J = 8.0, 1.5, H-6), 7.82 (1H, dd, J = 8.0, 1.5, H-15), 7.76 (1H, m, H-5), 7.73 (1H, m, H-7), 7.71 (1H, m, H-14), 7.64 (1H, ddd, J = 8.0, 7.0, 1.5, H-13), 7.33 (3H, overlapped, H-23–25), 7.10 (1H, dd, J = 8.0, 1.5, H-22, 26), 5.87 (1H, s, 20-OH), 4.56 (1H, d, J = 10.5, H-19), 3.84 (1H, d, J = 10.5, H-20), 2.57 (3H, s, H-27). ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 164.7 (C-2), 161.2 (C-10), 153.1 (C-18), 146.3 (C-16), 141.2 (C-21), 135.2 (C-14), 132.6 (C-8), 131.7 (C-3), 131.2 (C-6), 130.0 (C-4), 129.1 (C-5), 129.0 (C-24), 128.4 (CH-23, 25), 128.2 (C-7), 127.7 (C-13), 127.3 (C-12), 126.8 (C-15), 126.4 (C-22, 26), 121.0 (C-11), 74.2 (C-19), 70.2 (C-20), 37.4 (C-27). (+)-ESI-MS *m/z* 398 $[M + H]^+$.

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REFERENCES

1. X. M. Hou, Y. Hai, Y. C. Gu, C. Y. Wang, and C. L. Shao, *Curr. Med. Chem.*, **26**, 1 (2019).
2. X. M. Hou, R. F. Xu, Y. C. Gu, C. Y. Wang, and C. L. Shao, *Curr. Med. Chem.*, **22**, 3707 (2015).
3. H. Sun, C. J. Barrow, D. M. Sedlock, A. M. Gillum, and R. Cooper, *J. Antibiot.*, **47**, 515 (1994).
4. L. Rahbek, J. Breinholt, J. C. Frisvad, and C. Christophersen, *J. Org. Chem.*, **64**, 1689 (1999).
5. J. F. Liu, M. Kaselj, Y. Isome, J. Chapnick, B. L. Zhang, G. Bi, D. Yohannes, L. B. Yu, and C. M. Baldino, *J. Org. Chem.*, **70**, 10488 (2005).
6. C. M. Cui, X. M. Li, C. S. Li, H. F. Sun, S. S. Gao, and B. G. Wang, *Helv. Chim. Acta*, **92**, 1366 (2009).
7. F. H. Song, B. Ren, K. Yu, C. X. Chen, H. Guo, N. Yang, H. Gao, X. T. Liu, M. Liu, Y. J. Tong, H. Q. Dai, H. Bai, J. D. Wang, and L. X. Zhang, *Mar. Drugs*, **10**, 1297 (2012).
8. L. Liu, Y. Y. Zheng, C. L. Shao, and C. Y. Wang, *Mar. Life Sci. Technol.*, **1**, 60 (2019).
9. X. M. Hou, T. M. Liang, Z. Y. Guo, C. Y. Wang, and C. L. Shao, *Chem. Commun.*, **55**, 1104 (2019).
10. X. M. Hou, Y. Y. Li, Y. W. Shi, Y. W. Fang, R. Chao, Y. C. Gu, C. Y. Wang, and C. L. Shao, *J. Org. Chem.*, **84**, 1228 (2019).
11. S. Toshiyuki, O. Tomohiro, E. Shoji, K. Akikazu, Y. Eiji, and O. Yoshio, *Tetrahedron*, **54**, 7997 (1998).
12. J. P. Jang, J. H. Jang, N. K. Soung, H. M. Kim, S. J. Jeong, Y. Asami, K. S. Shin, M. R. Kim, H. Oh, B. Y. Kim, and J. S. Ahn, *J. Antibiot.*, **65**, 215 (2012).
13. Q. M. Feng, Y. Feng, T. Y. Zhang, H. F. Wang, M. Y. Zhang, Y. Y. Wu, G. Chen, Y. X. Zhang, and Y. H. Pei, *Nat. Prod. Res.*, 1478 (2019).