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BIOACTIVITY ISOCHROMENES FROM CIGAR TOBACCO-DERIVED ENDOPHYTIC FUNGUS *Aspergillus fumigatus*

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*Two new isochromenes, 1-(8-methoxy-3-methyl-1*H*-isochromen-6-yl)propan-1-one (**1**) and 3-hydroxy-1-(8-methoxy-3-methyl-1*H*-isochromen-7-yl)propan-1-one (**2**), along with five known ones, (**3–7**) were isolated from the fermentation products of a cigar tobacco-derived endophytic fungus* Aspergillus fumigatus*. Their structures were elucidated by spectroscopic methods, including extensive 1D and 2D NMR techniques. Compounds **1** and **2** were evaluated for their antibacterial activities against* Pseudomonas syringae *(the main pathogenic sources of tobacco angular spot disease). Compounds **1** and **2** showed activity with MIC_{50} values of 6.8 and 8.4 μg/mL, respectively, whereas the MIC_{50} value of the positive control (agricultural streptomycin) is 2.2 μg/mL. The efficiencies of **1** and **2** are lower than that of the positive control.*

Keywords: isochromenes, tobacco, endophytic fungus *Aspergillus fumigatus*, antibacterial activity.

Plant diseases have harmful effects on the growth and yield of crops. The attacking pathogens can cause serious losses to various crops worldwide. For main crops, their quality and yield are notably affected once they are infected by pathogenic bacteria, fungi, or viruses [1, 2]. Among the most plant pathogenic bacteria, *Pseudomonas syringae* is the main pathogen for angular spot disease, and can attack a wide range of hosts, such as tobacco, pepper, tomato, and cucumber [3–5]. For tobacco especially, angular spot disease outbreaks often result in losses as they reduce the yield and quality of the tobacco [6].

In agriculture, breeding resistant varieties, inducing plants resistance, improving cultivation, biological control, and chemical pesticides are the main methods of preventing tobacco diseases [7]. As compared with synthetic chemicals, natural products are highly promising prevention strategies owing to their low toxicity, with no residual effects [8, 9]. Therefore, they have attracted increasing attention from plant protection scholars, and have led more and more biological companies to commit to developing natural products into new pesticides [10, 11]. Among the numerous natural products, isochromenes are abundant in fungal and plant metabolites, and they are mainly derived from the polyketide pathways [12]. As one of the characteristic components of *Aspergillus* fungi, isochromenes also attract much attention from chemists and biologists owing to their diverse structures and biological properties [13–18].

Aspergillus is an important fungal genus containing economically important species, as well as pathogenic species of animals and plants. They can also produce a number of structurally complicated molecules with various biological activities [19–21]. As part of an ongoing program to screen active metabolites from *Aspergillus* fungi, we made a scale-up solid fermentation experiment using a selected cigar tobacco-derived strain *A. fumigatus*, and the phytochemical studies led to the isolation of two new (**1** and **2**) and five known (**3–7**) isochromenes. Details of the isolation, structural elucidation, and bioactivities of these metabolites are reported herein.

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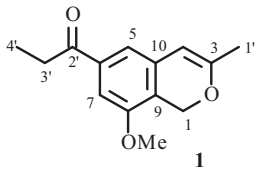
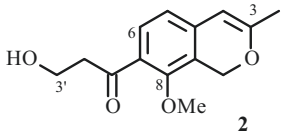
TABLE 1. ^{1}H and ^{13}C NMR Data of Compounds **1** and **2** ($CDCl_3$, δ, ppm, J/Hz)

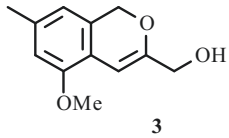
C atom	**1**		**2**	
	δ_H	δ_C	δ_H	δ_C
1	5.17 (s)	63.9 (CH_2)	5.17 (s)	63.2 (CH_2)
3	–	158.6 (C)	–	158.3 (C)
4	5.79 (d, J = 0.6)	100.2 (CH)	5.78 (d, J = 0.6)	100.6 (CH)
5	6.85 (d, J = 2.2)	116.6 (CH)	6.67 (d, J = 8.2)	113.5 (CH)
6	–	135.9 (C)	7.67 (d, J = 8.2)	128.5 (CH)
7	7.46 (d, J = 2.2)	112.1 (CH)	–	112.3 (C)
8	–	155.4 (C)	–	156.7 (C)
9	–	118.2 (C)	–	115.6 (C)
10	–	137.4 (C)	–	138.5 (C)
1′	1.98 (d, J = 0.6)	18.8 (CH_3)	2.00 (d, J = 0.6)	18.8 (CH_3)
2′	–	201.1 (C)	–	199.3 (C)
3′	3.44 (m)	31.9 (CH_2)	2.81 (t, J = 6.6)	39.6 (CH_2)
4′	1.41 (t, J = 7.2)	9.22 (CH_3)	3.66 (t, J = 6.6)	58.3 (CH_2)
MeO	3.77 (s)	56.2 (CH_3)	3.85 (s)	61.3 (CH_3)



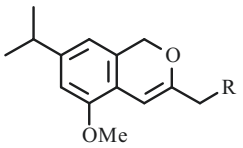
1



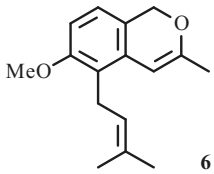
2



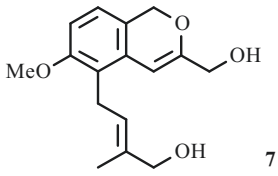
3



4, 5



6

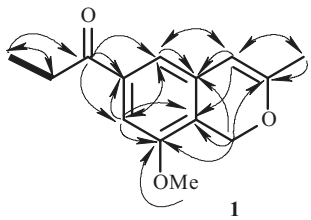


7

4: R = H; **5:** R = OH

The whole culture broth from the fermentation products of *A. fumigatus* was extracted with ethyl acetate. The extract was subjected repeatedly to column chromatography and preparative HPLC to afford compounds **1–7**, including two new isochromenes (**1**, **2**), and five known analogues (**3–7**). The new compounds were confirmed by the search of the newly updated sci-finder database (an electronic database for chemical structure published by the American Chemical Society). The known compounds, (5-methoxy-7-methyl-1*H*-isochromen-3-yl)methanol (**3**) [16], 7-isopropyl-5-methoxy-3-methyl-1*H*-isochromene (**4**) [17], (7-isopropyl-5-methoxy-1*H*-isochromen-3-yl)methanol (**5**) [17], versicolol B (**6**) [22], and oryzaein D (**7**) [23] were identified by the comparison of their spectroscopic data with those in the literature.

Compound **1** was obtained as a pale yellow gum. Its molecular formula $C_{14}H_{16}O_3$ was determined by positive HR-ESI-MS at *m/z* 255.0987 $[M + Na]^+$ (calcd for $C_{14}H_{16}NaO_3$, 255.0992), indicating seven degrees of unsaturation. The IR spectrum showed the absorption bands of carbonyl (at 1688 cm^{-1}), and aromatic groups (at 1622, 1545, and 1473 cm^{-1}). The UV spectrum showed absorption maxima at 275 and 342 nm, also suggesting the presence of an aromatic ring in the molecule. The ^{1}H and ^{13}C NMR spectra data (Table 1) showed signals assignable to a 1,2,3,5-tetrasubstituted benzene ring (C-5–C-10, H-5, and H-7), a pair of double bonds (C-3, C-4, and H-4), an oxidized methylene carbon (C-1 and H_2-1), a methyl group (C-1′ and H_3-1′), a propanoyl group ((-(C=O)CH_2CH_3, C-2′–C-4′, H_2-3′, H_3-4′) [24], and a methoxy group (δ_C 56.2 q and δ_H 3.77 s). According to the above NMR data, the oxidized aromatic quaternary carbon in double bonds (C-3) and oxidized methylene carbon (C-1) should be incorporated with a benzene ring to form an isochromen ring to support the seven degrees of unsaturation in the molecule [16, 17]. In addition, the existence of the isochromen core was supported by the HMBC correlations (Fig. 1) from H-1 to C-3, C-8, C-9, and C-10, from H-4 to C-3, C-5, C-9, and C-10, and from H-5 to C-4. The existence of the propanoyl group was supported by the HMBC correlations from H_3-4′ to C-2′, C-3′, from H_2-3′ to C-2′, C-4′, and ^{1}H–^{1}H COSY correlations of H_2-3′/H_3-4′.



1

Fig. 1. Key HMBC and ^{1}H–^{1}H COSY correlations of **1**.

As the isochromen skeleton was determined, the positions of the substituents (methyl, propanoyl, and methoxy groups) can also be determined by further analysis of its HMBC data (Fig. 1). The HMBC correlations from the H_3-1′ to C-3, C-4, from H-4 to C-1′ established that the methyl group was located at C-3. The methoxy group located at C-8 was clearly indicated by the HMBC correlations from the methoxy proton (δ_H 3.77 s) to C-8. Furthermore, the propanoyl group located at C-6 can also be determined by the HMBC correlations of H_2-3′ with C-6, and of H-5 and H-7 with C-2′. On the basis of the above evidence, the structure of **1** was established as shown, and gave the systematic name of 1-(8-methoxy-3-methyl-1*H*-isochromen-6-yl)propan-1-one.

Compound **2** was also obtained as a pale-yellow gum. The molecular formula was established as $C_{14}H_{16}O_4$, by the $[M + Na]^+$ ion at *m/z* 271.0945 (calcd for $C_{14}H_{16}NaO_4$, 271.0941) in HR-ESI-MS. The ^{1}H and ^{13}C NMR data (Table 1) of **1** and **2** showed high similarity. The obvious chemical shift differences resulted from the substituent groups' variation on the benzene ring, and the propanoyl group in **1** was replaced by a 3-hydroxypropanoyl group (OH-CH_2CH_2CO-, C-2′–C-4′, H_2-3′ and H_2-4′) [25] in **2**. The existence of the 3-hydroxypropanoyl group was also supported by the HMBC correlations from H_2-4′ to C-2′, C-3′, from H_2-3′ to C-2′, C-4′, and ^{1}H–^{1}H COSY correlations of H_2-3′/H_2-4′. In addition, the position of the 3-hydroxypropanoyl group at C-7 can be determined by the HMBC correlations from H_2-3′ to C-7, and from H-6 to C-2′. The positions of the methyl group at C-3 and the methoxy group at C-8 can also be determined by the HMBC correlations from H_3-1′ to C-3, C-4, and from methoxy proton (δ_H 3.85 s) to C-8 respectively. Thus, the structure of 3-hydroxy-1-(8-methoxy-3-methyl-1*H*-isochromen-7-yl)propan-1-one (**2**) was determined as shown.

As certain of the isochromens exhibit potential antibacterial activity [18, 26, 27], and the bacteria, *Pseudomonas syringae* pv. *angulata*, is the main cause of tobacco angular spot disease [6]. Compounds **1** and **2** were tested for their anti-*P. syringae* activity. The compounds were assayed for their antimicrobial activities in 96-well plates according to the literature [28, 29]. The results revealed that compounds **1** and **2** showed activity, with MIC_{50} values of 6.8 and 8.4 μg/mL, respectively, whereas the MIC_{50} value of the positive control (agricultural streptomycin) is 2.2 μg/mL.

EXPERIMENTAL

General Methods. UV spectra were obtained using a Shimadzu UV-1900 spectrophotometer. A Bio-Rad FTS185 spectrophotometer was used for scanning IR spectra. ^{1}H, ^{13}C, and 2D NMR spectroscopic data were recorded on a DRX-500 NMR spectrometer with TMS as the internal standard. ESI-MS and HR-ESI-MS analyses were measured on an Agilent 1290UPLC/6540 Q-TOF mass spectrometer. Semipreparative HPLC was performed on an Agilent 1260 preparative liquid chromatograph with Zorbax PrepHT GF (2.12 × 25 cm) or Venusil MP C_{18} (2.0 × 25 cm) columns. Column chromatography was performed using silica gel (200–300 mesh, Qingdao Marine Chemical, Inc., Qingdao, China), Lichroprep RP-18 gel (40–63 μm, Merck, Darmstadt, Germany), Sephadex LH-20 (Sigma-Aldrich, Inc, USA), or MCI gel (75–150 μm, Mitsubishi Chemical Corporation, Tokyo, Japan). Column fractions were monitored by TLC visualized by spraying with 5% H_2SO_4 in ethanol and heating.

Fungal Material. The culture of *Aspergillus fumigatus* was isolated from the leaves of cigar tobacco, collected from Gengma County, Lincang Prefecture, Yunnan Province, in 2022. The strain (YATAS-22-042) was identified by one of authors (Dr. Yin-Ke Li) based on the analysis of the ITS sequence. It was cultivated at room temperature for 7 days on potato dextrose agar at 28°C. Agar plugs were inoculated into 250-mL Erlenmeyer flasks each containing 100 mL potato dextrose broth and cultured at 28°C on a rotary shaker at 180 rpm for 5 days. Large-scale fermentation was carried out in 100 Fernbach flasks (500 mL) each containing 300 mL of medium (glucose 5%; peptone 0.15%; yeast 0.5%; KH_2PO_4 0.05%;

$MgSO_4$ 0.05% in 1.0 L of deionized water; pH 6.5 before autoclaving). Each flask was inoculated with 5.0 mL of cultured broth and incubated at 27°C for 20 days.

Extraction and Isolation. The whole culture broth of *A. fumigatus* was extracted four times with ethyl acetate (4 × 15 L) at room temperature and filtered. The crude extract (102.8 g) was applied to silica gel column chromatography, eluting with a $CHCl_3$–MeOH gradient system (9:1, 8:2, 7:3, 6:4, 5:5). Five fractions were obtained from the silica gel column and individually decolorized on MCI gel to yield fractions A–E. The further separation of fraction A (9:1, 18.2 g) by silica gel column chromatography, eluted with $CHCl_3$–$(CH_3)_2CO$ (9:1, 8:2, 7:3, 6:4, 1:1), yielded mixtures A1–A5. Fraction A2 (8:2, 3.54 g) was subjected to RP-18 column chromatography (MeOH–H_2O 20:80–80:20 gradient) and HPLC to give **6** (22.6 mg). Fraction A3 (7:3, 2.38 g) was subjected to RP-18 column chromatography (MeOH–H_2O, 20:80–70:30 gradient) and HPLC to give **1** (14.4 mg), **4** (16.0 mg), and **7** (18.3 mg). The further separation of Fr. B (8:2, 13.5 g) by silica gel column chromatography, eluted with $CHCl_3$–$(CH_3)_2CO$ (8:2, 7:3, 6:4, 1:1, 4:6), yielded mixtures B1–B5. Fraction B3 (6:4, 3.06 g) was subjected to RP-18 column chromatography and HPLC (MeOH–H_2O, 20:80–60:40 gradient) to give **2** (14.2 mg), **3** (16.1 mg), and **5** (12.2 mg).

Antibacterial Assays. The strain of bacteria (*Pseudomonas syringae* pv. *angulata*) was obtained from Yunnan Academy of Tobacco Agricultural Sciences. The antibacterial activities were tested by a serial dilution technique using 96-well microtiter plates [28, 29], using agricultural streptomycin (a commercial product for plant bacteria disease in China) as a positive control. The tested compounds and positive control were dissolved in DMSO to give a stock solution.

1-(8-Methoxy-3-methyl-1*H*-isochromen-6-yl)propan-1-one (1), pale yellow gum, $C_{14}H_{16}O_3$. UV (MeOH, λ_{max}, nm) (log ε): 215 (3.90), 275 (3.78), 342 (3.66). IR (ν_{max}, cm^{-1}): 3056, 2942, 2867,1688, 1622, 1545, 1473, 1368, 1239, 1170, 1058, 860. For ^1H (500 MHz) and ^{13}C (125 MHz) NMR data, see Table 1. ESI-MS *m/z* 255 $[M + Na]^+$; HR-ESI-MS *m/z* 255.0987 (calcd for $C_{14}H_{16}NaO_3$, 255.0992).

3-Hydroxy-1-(8-methoxy-3-methyl-1*H*-isochromen-7-yl)propan-1-one (2), $C_{14}H_{16}O_4$. UV (MeOH, λ_{max}, nm) (log ε): 215 (3.84), 272 (3.83), 340 (3.68). IR (ν_{max}, cm^{-1}): 3395, 3062, 2948, 2860, 1685, 1624, 1548, 1470, 1362, 1247, 1158, 1066, 848. For ^1H (500 MHz) and ^{13}C (125 MHz) NMR data, see Table 1. ESI-MS *m/z* 271 $[M + Na]^+$; HR-ESI-MS *m/z* 271.0945 (calcd for $C_{14}H_{16}NaO_4$, 271.0941).

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REFERENCES

1. K. Basaid, B. Chebli, E. H. Mayad, J. N. Furze, R. Bouharroud, F. Krier, M. Barakate, and T. Paulitz, *Int. J. Pest. Manag*., 67 (2), 155 (2021).
2. D. B. Collinge, D. F. Jensen, M. Rabiey, S. Sarrocco, M. W. Shaw, and R. H. Shaw, *Plant. Pathol*., **71** (5), 1024 (2022).
3. A. Ahmet, A. Sahika, O. Hatice, G. Ayse, and A. Mustafa, *Sci. Hortic-Amsterdam*, **282** (1), 110005 (2021).
4. M. Sato, K. Watanabe, and Y. Sato, *J. Gen. Plant. Pathol.*, **67**, 303 (2001).
5. P. Yang, L. J. Zhao, Y. G. Gao, and Y. Xia, *Plants*, **12** (9), 1765 (2023).
6. G. Diana, *Diseases of Tobacco: Bacteria*, Informatore Fitopatologico, 1994, pp. 29–32.
7. C. Verma, S. Jandaik, N. Kashyap, B. K. Gupta, and A. Kerketta, *Int. J. Chem. Stud*., **8** (4), 2570 (2020).
8. K. H. Basaid, B. Chebli, E. H. Mayad, J. N. Furze, R. Bouharroud, F. Krier, M. Barakate, and T. Paulitz, *Int. J. Pest. Manag*., **67** (2), 155 (2021).
9. M. F. Jimenez-Reyes, H. Carrasco, A. Olea, and E. Silva-Moreno, *J. Chil. Chem. Soc*., **64** (2), 4459 (2019).
10. B. A. Lorsbach, T. C. Sparks, R. M. Cicchillo, N. V. Garizi, D. R. Hahn, and K. G. Meyer, *Pest. Manag. Sci*., **75** (9), 2301 (2019).
11. T. C. Sparks and R. J. Bryant, *Pest. Manag. Sci*., **78** (2), 399 (2022).

12. D. Engelmeier, F. Hadacek, O. Hofer, G. Lutz-Kutschera, M. Nagl, G. Wurz, and H. Greger, *J. Nat. Prod*., **67**, 19 (2004).
13. S. Kiran, D. Khan, H. Ullah, N. Sahab, and S. Khan, *Nat. Prod. Res*., **37** (14), 2390 (2023).
14. A. Saeed, *Eur. J. Med. Chem*., **116**, 290 (2016).
15. S. Pal, V. Chatare and M. Pal, *Curr. Org. Chem*., **15**, 782 (2011).
16. F. X. Yang, J. M. Dai, H. Y. Liu, Q. L. Mi, J. Wang, J. D. Zhang, X. M. Li, W. G. Wang, M. Zhou, Y. K. Li, and Q. F. Hu, *Nat. Prod. Res*., **37** (10), 1608 (2023).
17. Q. F. Hu, L. F. Zhang, G. H. Zhang, M. F. Bao, Y. K. Li, M. Dong, Y. P. Wu, G. Du, and G. H. Kong, *Heterocycles*, **104** (9), 1661 (2022).
18. H. Y. Liu, F. X. Yang, J. M. Dai, M. J. Liang, W. Xiong, Q. L. Mi, X. M. Li, K. Wang, L. Deng, Q. F. Hu, and J. D. Zhang, *Chem. Nat. Compd*., **59**, 41 (2023).
19. J. M. Dai, Q. L. Mi, X. M. Li, D. Gang, G. Y. Yang, J. D. Zhang, J. Wang, Y. K. Li, H. Y. Yang, D. Miao, Z. J. Li, and Q. F. Hu, *Phytochemistry*, **205**, 113485 (2023).
20 F. X. Yang, H. Y. Liu, Z. J. Li, Q. L. Mi, X. M. Li, L. M. Zhang, G. Y. Yang, Y. K. Li, W. G. Wang, M. Zhou, X. W. Ma, and Q. F. Hu, *ACS Agric. Sci. Technol*., **3**, 131 (2023).
21. F. X. Yang, Y. Y. Ma, Y. P. Wu, G. K. Zhao, Y. P. Li, Z. J. Li, X. M. Li, Y. K. Li, W. G. Wang, M. Zhou, G. H. Kong, and Q. F. Hu, *Pestic. Biochem. Phys*., **196**, 105613 (2023).
22. M. Zhou, J. Lou, Y. K. Li, Y. D. Wang, K. Zhou, B. K. Ji, W. Dong, X. M. Gao, G. Du, and Q. F. Hu, *Arch. Pharm. Res*., **40** (1), 32 (2017).
23. M. Zhou, K. Zhou, P. He, K. M. Wang, R. Z. Zhu, Y. D. Wang, W. Dong, G. P. Li, H. Y. Yang, Y. Q. Ye, G. Du, X. M. Li, and Q. F. Hu, *Planta Med*., **82**, 414 (2016).
24. M. J. Hao, P. N. Chen, H. J. Li, F. Wu, G. Y. Zhang, Z. Z. Shao, X. P. Liu, W. Z. Ma, J. Xu, T. Mahmud, and W. J. Lan, *Front. Microbiol*., **13**, 947226 (2022).
25. G. Y. Yang, J. M. Dai, Z. J. Li, J. Wang, F. X. Yang, X. Liu, J. Li, Q. Gao, X. M. Li, Y. K. Li, W. G. Wang, M. Zhou, and Q. F. Hu, *Arch. Pharm. Res*., **45**, 572 (2022).
26. P. Manivel, S. M. Roopan, D. P. Kumar, and F. N. Khan, *Phosphorus. Sulfur*., **184** (10), 2576 (2009).
27. Z. H. Shu, J. H. Zhou, J. Y. Li, Y. L. Cheng, H. Liu, D. H. Wang, and Y. Zhou, *J. Org. Chem*., **85** (19), 12097 (2020).
28. L. P. Chi, X. M. Li, Y. P. Wan, Y. H. Li, X. Li, and B. G. Wang, *Chem. Biodiv*., **18**, e2100512 (2021).
29. C. G. Pierce, P. Uppuluri, A. R. Tristan, F. L. Wormley Jr. E. Mowat, G. Ramage, and J. L. Lopez-Ribot, *Nat. Protoc*., **3**, 1494 (2008).