DOI 10.1007/s10600-023-04089-2
Chemistry of Natural Compounds, Vol. 59, No. 4, July, 2023

ANTIBACTERIAL AROMATIC SESQUITERPENES FROM THE LEAVES OF *Nicotiana tabacum*

Jia-Meng Dai,[1] Wen Xiong,[1] Wen-Yuan Wang,[1] Hui-Ping Wang,[2] Wei Zhao,[1] Yong Li,[1] Yin-Ke Li,[3] Qiu-Fen Hu,[1,3] Wei-Guang Wang,[1,3] Shan-Zhai Shang,[1*] and Jie-Yun Cai[2*]

*Two new aromatic sesquiterpenes, 1-isopropyl-5-methoxy-3,7-dimethylnaphthalene (**1**) and 3-hydroxymethyl-1-isopropyl-5-methoxy-7-methylnaphthalene (**2**), as well as five known sesquiterpenes (**3**–**7**), were isolated from the leaves of* Nicotiana tabacum *(a mutant tobacco for solanesol synthesis gene knockout by CRISPR-Cas9 gene editing). Their structures were determined by spectroscopic methods, including extensive 1D and 2D NMR techniques. Compounds **1** and **2** were tested for their anti-methicillin-resistant* Staphylococcus aureus *(anti-MRSA) activity. The results revealed that compounds **1** and **2** showed good inhibition with IZD of 12.8 ± 2.0 and 15.2 ± 2.2 mm, respectively. In addition, compounds **1** and **2** have a weak floral fragrance, and they have the potential for use as an essence for cigarettes.*

Keywords: *Nicotiana tabacum* L., sesquiterpenes, anti-MRSA activity, essence.

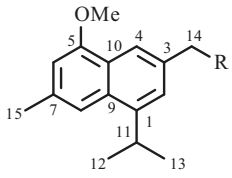
Sesquiterpenes are an important class of natural products possessing three isoprene-derived units widely distributed across plants, marine organisms, and microbes [1, 2], and their basic framework can be classified as acyclic, monocyclic, bicyclic, tricyclic, and multicyclic based on the number of carbon rings [3]. The discovery of artemisinin had stimulated research on sesquiterpenes for druggable analogs [4, 5], and many sesquiterpenes also showed a wide range of biological activity such as antitumor [6], antibiotic [7], antiviral [8], immunosuppressive [9], insecticidal [10], antifungal [11], and similar.

Nicotiana tabacum L. (Tobacco) is a cultivated small robust annual branched herb in the Solanaceae (nightshade) family. It is commercially cultivated in many countries, and its leaves are used as raw material to be processed into tobacco [12]. The economic importance of tobacco has encouraged researchers to investigate this species in detail, and approximately 9600 chemicals have been reported in tobacco and cigarette smoke [13]. Moreover, the biodiversity of phytochemicals in tobacco, such as alkaloids, terpenoids, flavonoids, lignans, phenolic acids and coumarins, may provide resources for the future development of medicines as well as agrochemicals [13, 14]. Sesquiterpenes are an important kind of metabolite in tobacco, and more than 80 sesquiterpenes have been reported [14]. The sesquiterpenes from *N. tabacum* have strong biological activity including cytotoxicity [15], antiviral [16, 17] and antibacterial activity [18], and ecological roles in the *Nicotiana* species, including as phytoalexins or in plant-herbivore interactions [19].

1) Yunnan Key Laboratory of Tobacco Chemistry, China Tobacco Yunnan Industrial Co., Ltd., 650231, Kunming, P. R. China, e-mail: jszxtg_2015@163.com; shangshanzhai1985@163.com; 2) Yunnan Tobacco Quality Supervision and Test Station, Kunming, 650106, Yunnan, P. R. China, e-mail: caijykm@126.com; 3) Key Laboratory of Chemistry in Ethnic Medicinal Resources, Yunnan Minzu University, 650031, Kunming, P. R. China. Published in *Khimiya Prirodnykh Soedinenii*, No. 4, July–August, 2023, pp. 581–584. Original article submitted October 13, 2022.

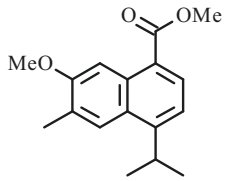
TABLE 1. ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR Data of Compounds **1** and **2** ($CDCl_3$, δ, ppm, J/Hz)

C atom	**1**		**2**	
	δ_H	δ_C	δ_H	δ_C
1	–	142.4 (C)	–	142.6 (C)
2	7.12 (d, J = 2.2)	128.8 (CH)	7.13 (d, J = 2.2)	129.4 (CH)
3	–	134.9 (C)	–	133.5 (C)
4	7.82 (d, J = 2.2)	118.6 (CH)	7.91 (d, J = 2.2)	119.3 (CH)
5	–	156.3 (C)	–	156.6 (C)
6	6.65 (d, J = 1.8)	107.0 (CH)	6.67 (d, J = 1.8)	106.8 (CH)
7	–	136.6 (C)	–	136.5 (C)
8	7.46 (d, J = 1.8)	115.4 (CH)	7.49 (d, J = 1.8)	115.6 (CH)
9	–	132.3 (C)	–	132.0 (C)
10	–	126.5 (C)	–	126.9 (C)
11	3.21 (m)	32.2 (CH)	3.23 (m)	32.3 (CH)
12, 13	1.33 (d, J = 6.8)	23.4 (CH_3)	1.32 (d, J = 6.8)	23.5 (CH_3)
14	2.48 (s)	21.2 (CH_3)	4.61 (s)	66.3 (CH_2)
15	2.37 (s)	20.7 (CH_3)	2.36 (s)	20.5 (CH_3)
5-OMe	3.82 (s)	56.3 (CH_3)	3.81 (s)	56.3 (CH_3)

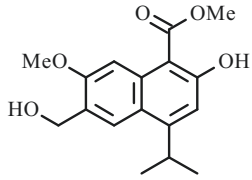


1, 2

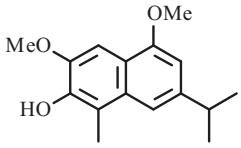
1: R = H; **2:** R = OH



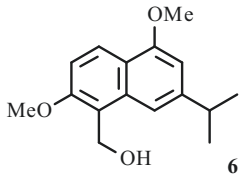
3



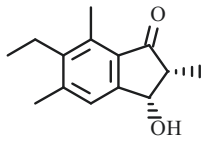
4



5



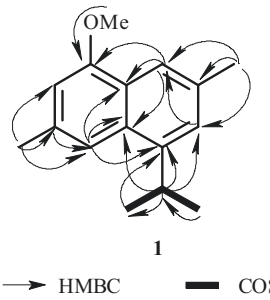
6



7

In recent years, gene-editing technology has been widely used in crop cultivar improvement [20], and a series of *N. tabacum* mutants have been cultivated by gene-editing technology. Because the secondary metabolism of plants is affected by the changes of genes [20, 21], the gene-editing mutants of tobacco provide a new sample source for the discovery of active metabolites. To discover more novel bioactive sesquiterpenes from *N. tabacum*, we focused on the leaves of YNZY-21-28 (a mutant tobacco for solanesol synthesis gene knockout by CRISPR-Cas9 gene editing) and isolated two new (**1** and **2**) and five known (**3–7**) aromatic sesquiterpenes. The new compounds were elucidated using spectroscopic methods, whereas the known compounds were identified by comparison of NMR data with the literature. Compounds **1** and **2** were also evaluated for their anti-methicillin-resistant *Staphylococcus aureus* (anti-MRSA) activity. Herein, we describe the isolation, structural determination, and bioassay of the above compounds.

A 95% aq. EtOH extract prepared from the leaves of tobacco was subjected repeatedly to column chromatography and preparative HPLC to afford two new sesquiterpenes, 1-isopropyl-5-methoxy-3,7-dimethylnaphthalene (**1**) and 3-hydroxymethyl-1-isopropyl-5-methoxy-7-methylnaphthalene (**2**), together with five known sesquiterpenes (**3–7**). The structures of compounds **1–7** were shown in Fig. 1, and the ^{1}H and ^{13}C NMR data of **1** and **2** were listed in Table 1. The new compounds were confirmed by searching the newly updated sci-finder database (an electronic database for chemical structure published by the American Chemical Society). The known compounds were identified as methyl 4-isopropyl-7-methoxy-6-methyl-naphthalene-1-carboxylate (**3**) [15], methyl 2-hydroxy-6-(hydroxymethyl)-4-isopropyl-7-methoxy-naphthalene-1-carboxylate (**4**) [15], 7-isopropyl-3,5-dimethoxy-1-methylnaphthalen-2-ol (**5**) [22], (3-isopropyl-1,6-dimethoxy-naphthalen-5-yl)-methanol (**6**) [22], and nicosesquiterpene A (**7**) [23], by comparison with the literature.



1

HMBC COSY

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

Compound **1** was obtained as a pale-yellow gum, and it has the molecular formula $C_{16}H_{20}O$, which was established using HR-ESI-MS at *m/z* 251.1418 $[M + Na]^+$ (calcd for 251.1412), suggesting seven degrees of unsaturation. The UV spectrum showed absorption maxima at 215, 252, and 305 nm, and the IR spectrum showed absorption bands at 1618, 1569, and 1457 cm^{-1}, indicating the presence of hydroxy and aromatic rings. Its ^{1}H, ^{13}C, and DEPT NMR spectra displayed signals for 16 carbons and 20 hydrogen atoms. These signals correspond to one tetra-substituted naphthalene nucleus (C-1–C-10, H-2, H-4, H-6, and H-8) [15, 22], an isopropyl group (C-12–C-13, H-11, and H_6-12, 13) [24], two methyl groups (C-14, C-15, H_3-14, and H_3-15), and one methoxy group (δ_C 56.3 s, δ_H 3.82 s). The existence of tetra-substituted naphthalene can also be confirmed by the HMBC correlations from H-8 to C-1, C-9, and C-10, from H-4 to C-5, and C-9, 10, whereas the existence of the isopropyl group was verified by the ^{1}H–^{1}H COSY correlations (Fig. 1) of H-11/H_6-12, 13, and the HMBC correlations from H_6-12, 13 to C-11, from H-11 to C-12, 13. In addition, the HMBC correlations from H-11, H_6-12, 13 to C-1, indicated that the isopropyl group was located at C-1. Two methyl groups located at C-3 and C-7 were also confirmed by the HMBC correlations between H_3-14 and C-2/C-3/C-4, and between H_3-15 and C-6/C-7C-8, respectively. Furthermore, the methoxy group located at C-5 was confirmed on account of the HMBC correlations from methoxy proton (δ_H 3.82 s) to C-5. Thus, the structure of **1** was elucidated, and given the systematic name of 1-isopropyl-5-methoxy-3,7-dimethylnaphthalene.

3-Hydroxymethyl-1-isopropyl-5-methoxy-7-methylnaphthalene (**2**) was also isolated as a pale-yellow gum and it gave an $[M + Na]^+$ peak at *m/z* 267.1365, consistent with a molecular formula of $C_{16}H_{20}O_2$. Its ^{1}H and ^{13}C NMR spectroscopic data were similar to those of **1**, which suggested that **2** should be structurally related to **1**. The obvious chemical shift differences resulted from a methyl group in **1** being replaced by a hydroxymethyl group (C-14, H_2-14) in **2**, and the hydroxymethyl group located at C-3 was supported by the HMBC correlations from H_2-14 to C-2/C-3/C-4. In addition, the position of the methyl group located at C-7, the isopropyl group at C-1, and the methoxy group at C-5 can also be determined by further analysis of its HMBC correlations. Thus, the structure of **2** was determined as shown.

Because certain of the sesquiterpenes exhibit potential antibacterial activity [25, 26], compounds **1** and **2** were screened for anti-MRSA activity [27] according to an arbitrary criterion with a inhibition zone diameter (IZD) as follows: very weak inhibition (with an IZD of 6–8 mm), weak inhibition (with an IZD of 8–12 mm), good inhibition (with an IZD of 12–16 mm), and strong inhibition (with an IZD of > 16 mm). The IZD of the positive control (vancomycin) was 32 mm and the negative control was zero. The results revealed that compounds **1** and **2** showed good inhibition with IZDs of 12.8 ± 2.0 and 15.2 ± 2.2 mm, respectively. In addition, compounds **1** and **2** also have a weak floral fragrance, and they have the potential for use as an essence for cigarettes.

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 1290 UPLC/6540 Q-TOF mass spectrometer. Semipreparative HPLC was performed on an Agilent 1260 preparative liquid chromatograph with Zorbax PrepHT GF (2.12 cm × 25 cm) or Venusil MP C_{18} (2.0 cm × 25 cm) columns. Column chromatography was performed using silica gel (200–300 mesh, Qingdao Marine Chemical, Qingdao, China), Lichroprep RP-18 gel (40–63 μm, Merck, Darmstadt, Germany), Sephadex LH-20 (Sigma-Aldrich, 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.

Plant Material. The tobacco (YNZY-21-28, a gene editing mutant for solanesol synthesis gene knockout by CRISPR-Cas9 gene editing, cultivated by Yunnan China Tobacco Industry) was planted in a greenhouse in 2022 at Kunming, Yunnan Province. The tobacco leaves were picked at the mature stage, and the voucher specimen (YNZY-22-03) was deposited in the Key Laboratory of Chemistry in Ethnic Medicinal Resources, Yunnan Minzu University, P. R. China.

Extraction and Isolation. The air dried tobacco leaves (2.8 kg) were crushed to 30 mesh, and were extracted four times with 95% aqueous EtOH (4 × 6 L) at room temperature and filtered. The extract (106 g) was applied to silica gel (200–300 mesh) column chromatography, eluting with a $CHCl_3$–CH_3OH gradient system (10:0, 9:1, 8:2, 7:3, 6:4, 5:5), to give six fractions A–F. Further separation of Fr. B (9:1, 18.4 g) by silica gel column chromatography, eluted with $CHCl_3$–$(CH_3)_2CO$ (9:1–2:1), yielded subfractions B1–B6. Subfraction B1 (9:1, 4.26 g) was subjected to silica gel column chromatography using petroleum ether–acetone and semipreparative HPLC (64% MeOH–H_2O, flow rate 12 mL/min) to give **1** (14.8 mg) and **3** (15.2 mg). Subfraction B2 (8:2, 3.46 g) was subjected to silica gel column chromatography using petroleum ether–acetone and semipreparative HPLC (58% MeOH–H_2O, flow rate 12 mL/min) to give **2** (10.6 mg), **4** (12.2 mg), **5** (13.5 mg), **6** (14.0 mg), and **7** (12.9 mg).

1-Isopropyl-5-methoxy-3,7-dimethylnaphthalene (1), $C_{16}H_{20}O$ was obtained as a pale-yellow gum with a weak floral fragrance. UV (MeOH, λ_{max}, nm) (log ε): 305 (3.73), 252 (3.58), 215 (3.96). IR (KBr, ν_{max}, cm^{-1}): 3072, 2960, 1618, 1569, 1457, 1342, 1268, 1256, 1160, 884. For ^1H and ^{13}C NMR data ($CDCl_3$, 500 and 125 MHz, respectively), see Table 1. ESI-MS *m/z* 251 $[M + Na]^+$; HR-ESI-MS *m/z* 251.1418 $[M + Na]^+$ (calcd for $C_{16}H_{20}NaO$, 251.1412).

3-Hydroxymethyl-1-isopropyl-5-methoxy-7-methylnaphthalene (2), $C_{16}H_{20}O_2$ was obtained as a pale-yellow gum with a weak floral fragrance. UV (MeOH, λ_{max}, nm) (log ε): 310 (3.69), 255 (3.62), 215 (3.93). IR (KBr, ν_{max}, cm^{-1}): 3410, 3068, 2964, 1616, 1562, 1446, 1357, 1265, 1164, 910. For ^1H and ^{13}C NMR data ($CDCl_3$, 500 and 125 MHz, respectively), see Table 1. ESI-MS *m/z* 267 $[M + Na]^+$; HR-ESI-MS *m/z* 267.1365 $[M + Na]^+$ (calcd for $C_{16}H_{20}NaO_2$, 267.1361).

Anti-MRSA Agar Disk Diffusion Assay. The MRSA strain ZR11 was clinically isolated from infectious samples of critically ill patients in the Clinical Laboratory of the First People′s Hospital of Yunnan Province, and confirmed by a standard cefoxitin disk diffusion test following CLSI standard procedures [27]. The anti-MRSA activity of the compounds was evaluated via the disk diffusion method. The ZR11 strain was inoculated in Mueller Hinton Broth and incubated at 37°C for 24 h. The turbidity of bacterial suspension was adjusted to 0.5 McFarland standard, which is equal to 1.5×10^8 colony-forming units/mL. Sterile filter paper disks (6 mm) were impregnated with 20 μL (50 μg) of each compound and placed on inoculated Mueller Hinton agar containing bacterial suspension, adjusted to 0.5 McFarland standard. The commercially available disks containing 30 μg vancomycin were used as a positive control whereas disks without samples (5% DMSO) acted as a negative control. The inhibition zones including the diameter of the disk (mm) were measured and compared after incubation at 37°C for 24 h. The tests were carried out in triplicate for each sample.

ACKNOWLEDGMENT

This project was supported financially by the Foundation of Yunnan Tobacco Company (No. 2021530000241009), the Foundation of Yunnan Tobacco Industry Co. Ltd. (No. JB2022XY03), and the Foundation of Yunnan Innovative Research Team (2019HC020).

REFERENCES

1. H. Z. Shu, C. Peng, L. Bu, L. Guo, F. Liu, and L. Xiong, *Phytochemistry*, **192**, 112927 (2021).
2. B. Chopra and A. K. Dhingra, *Phytother. Res*., **35** (9), 4660 (2021).
3. D. E. Cane, *Compr. Nat. Prod. Chem*., **2**, 155 (1999).
4. K. M. Liu, H. L. Zuo, G. G. Li, H. Yu, and Y. J. Hu, *Pharmacol. Res*., **159**, 105048 (2020).
5. X. J. Cai, W. D. Guan, Q. H. Ma, S. L. Qin, and X. H. Wang, *Drugs Clinic*., **7** (3), 653 (2022).
6. P. Georgantea, E. Ioannou, E. Evain-Bana, D. Bagrel, N. Martinet, C. Vagias, and V. Roussis, *Tetrahedron*, **47** (23), 3262 (2016).

7. J. Bezerra, F. C. Rodrigues, R. Cruz, L. Silva, and M. Braga, *Appl. Sci*., **10** (2), 631 (2020).
8. E. Cenci, F. Messina, E. Rossi, F. Epifano, and M. C. Marcotullio, *Nat. Prod. Commun*., **7** (2), 143 (2012).
9. Q. Dai, F. L. Zhang, Z. H. Li, J. He, and T. Feng, *J. Fungi*., **7** (12), 1052 (2021).
10. I. C. Barreto, A. S. de Almeida, and J. G. Sena Filho, *Molecules*, **26** (21), 6405 (2021).
11. C. C. Wu, S. L. Huang, C. H. Ko, and H. T. Chang, *Molecules*, **27** (7), 2136 (2022).
12. R. Vagg and S. Chapman, *Addiction*, **100**, 701 (2015).
13. A. Rodgman and T. A. Perfetti, *The Chemical Components of Tobacco and Tobacco Smoke*, Second Edition, CRC Press, Taylor and Francis Group, Boca Raton, Florida, 2013.
14. A. R. Jassbi, S. Zare, M. Asadollahi, and M. C. Schuman, *Chem. Rev*., **117**, 12227 (2017).
15. P. S. Yang, S. Y. Tang, C. B. Liu, L. Ye, F. M. Zhang, P. He, Z. H. Liu, Y. K. Chen, M. M. Miao, Q. P. Shen, and J. Q. Wang, *J. Asian. Nat. Prod. Res*., **21** (2), 109 (2019).
16. Q. P. Shen, X. M. Xu, C. B. Liu, W. Zhao, N. J. Xiang, Y. K. Chen, M. M. Miao, Z. H. Liu, and G. Y. Yang, *Nat. Prod. Res*., **30** (22), 2545 (2016).
17. S. Z. Shang, W. Zhao, J. G. Tang, X. M. Xu, H. D. Sun, J. X. Pu, Z. H. Liu, M. M. Miao, Y. K. Chen, and G. Y. Yang, *Fitoterapia*, **108**, 1 (2016).
18. J. Chappell and R. Nable, *Plant. Physiol*., **85**, 469 (1987).
19. N. Song, L. Ma, W. G. Wang, H. H. Sun, L. Wang, I. T. Baldwin, and J. S. Wu, *J. Exp. Bot*., **70** (20), 5895 (2019).
20. D. L. Van Tassel, O. Tesdell, B. Schlautman, M. J. Rubin, L. R. De Haan, T. E. Crews, and A. S. Krug, *Front. Plant. Sci*., **11**, 789 (2020).
21. 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).
22. S. Z. Shang, W. Zhao, J. G. Tang, J. X. Pu, D. L. Zhu, L. Yang, H. D. Sun, G. Y. Yang, and Y. K. Chen, *Phytochem. Lett*., **17**, 173 (2016).
23. Q. P. Shen, X. M. Xu, L. Li, W. Zhao, N. J. Xiang, G. Y. Yang, Y. K. Chen, M. M. Miao, C. B. Liu, and Z. H. Liu, *Chin. Chem. Lett*., **27** (5), 753 (2016).
24. G. Y. Yang, J. M. Dai, Q. L. Mi, Z. J. Li, Xue-Mei Li, J. D. Zhang, J. Wang, Y. K. Li, W. G. Wang, M. Zhou, and Q. F. Hu, *Phytochemistry*, **198**, 113137 (2022).
25. X. Y. Liu, X. X. Fu, Y. Y. Li, Z. H. Xiong, B. T. Li, and W. W. Peng, *Nat. Prod. Res*., **35** (23), 4887 (2021).
26. G. H. Ma, K. X. Chen, L. Q. Zhang, and Y. M. Li, *Med. Chem. Res*., **28**, 1339 (2019).
27. Clinical and Laboratory Standards Institute, *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically*, Approved Standard, Vol. 32, Clinical and Laboratory Standards Institute, Wayne, PA, USA, 9th edition, 2012.