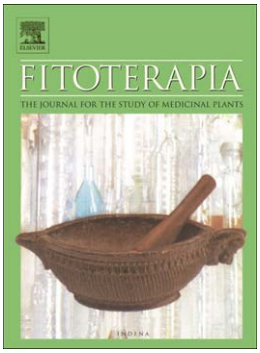

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Five New Lactone Derivatives from the Stems of *Dendrobium nobile*

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Abstract–Five new lactone derivatives decumbic acids A and B (**1** and **2**), (-)-decumbic acid (**3a**), (-)- and (+)-dendrolactone (**4a** and **4b**) together with four known compounds (**3b** and **5**–**7**) were isolated from the stems of *Dendrobium nobile*. Their structures were elucidated using comprehensive spectroscopic methods. Compounds **3a** and **3b**, **4a** and **4b** were isolated as two pair of enantiomers by chiral HPLC. The absolute configurations of **1**, **2**, **3a**, **4a** and **4b** were determined by optical rotation and X-ray crystallographic analysis. The inhibitory activities of all compounds against nine phytopathogenic fungi and three cancer cell lines were evaluated.

Keywords: Dendrobium nobile; butyrolactone; cytotoxic; antifungal

1. Introduction

The genus *Dendrobium* (Orchidaceae) is represented by about 1100 species of herbal plant, which are mainly distributed in the southwestern distributed throughout Asia, Europe and Australia. The stems of several *Dendrobium* species are used as a traditional medicine for the treatment of diabetes, fever, chronic atrophic gastritis, and skin aging diseases [1–6]. Our previous study on *Dendrobium nobile*, one of the species in southern China, has led to the isolation of 23 phenanthrene derivatives and five bibenzyl derivatives, some of the isolated products showed cytotoxic or antifungal activities [7]. In our ongoing investigation of natural antifungal and cytotoxic products, five new lactone derivatives decumbic acids A and B (**1** and **2**), (-)-decumbic acid (**3a**), (-)- and (+)-dendrolactone (**4a** and **4b**) together with four known compounds (Fig. 1), decumbic acid (**3b**) [8], 4-(3-hydroxyphenyl)-2-butanone (**5**) [9], 3-hydroxy-1(3-methoxy-4-hydroxyphenyl)-propan-1-one (**6**) [10] and 3',4',5',-trimethoxycinnamyl acetate (**7**) [11] were isolated from the stems of *D. nobile*. All compounds were tested for their antifungal activity against nine phytopathogenic fungi and cytotoxic activity on three cancer cell lines.

2. Experimental

2.1. General experimental procedures

IR spectra were recorded on a Nicolet 6700 spectrophotometer. Optical rotations were measured on a JASCO P-1020 digital polarimeter. Semi-Preparative HPLC was performed on an Agilent 1260 LC series with a DAD detector using an Agilent Eclipse XDB-C18 column (9.4 × 250 mm, 5 μm) and an ULTRON ES-OVM column (150 × 10 mm, 5 μm). NMR spectra were recorded on a Bruker AV spectrometer (400 MHz for ^{1}H and 100 MHz for ^{13}C). TMS was used as an internal standard. HRESIMS spectra were measured on a Q-TOF Ultima Global GAA076 LC mass spectrometer. Silica gel (Qing Dao Hai Yang Chemical Group Co.; 200–300 mesh) were used for column chromatography (CC). Precoated silica gel plates (Yan Tai Zi Fu Chemical Group Co.; G60, F-254) were used for thin layer chromatography (TLC). X-ray diffraction data were collected on an Aglient Technologies Gemini A Ultra system.

2.2. Plant material

The stems of *D. nobile* were provided by the Hainan Boying Orchid Industrial Development Co., LTD in June 2014. A voucher specimen (No. GFM20140612) has been deposited at the Key Laboratory of Tropical Chemistry of Medicinal Plant of Ministry of Education, Hainan Normal University (Hainan, China).

2.3. Extraction and isolation

The air-dried and powdered stems (5 kg) of *D. nobile* were extracted with 70% EtOH (3 × 20 L, 5 days each) at room temperature. After concentration under reduced pressure, the water-soluble residue was partitioned successively with petroleum ether and EtOAc. The EtOAc extract (95 g) was separated using a silica gel column chromatography (CC) (petroleum ether, EtOAc, MeOH v/v, gradient) to generate seven fractions (Frs. 1−7). Frs. 2 (17 g) was applied to silica gel CC eluted with petroleum ether-EtOAc (from 10:1 to 1:1) to afford four subfractions (2a–2d). Subfraction 2b was further purified by using Semi-Preparative HPLC (CH_3CN/H_2O, 30 : 70 *v*/*v*) to obtain **1** (10 mg), **2** (23 mg) and **3** (50 mg). Compound **3** further separated by using Semi-Preparative HPLC (CH_3CN/H_2O, 15 : 85 *v*/*v*, ULTRON ES-OVM column) to obtain **3a (**18 mg**)** and **3b** (20 mg). Subfraction 2c were further separated by Semi-Preparative HPLC (CH_3CN/H_2O, 35:65 *v*/*v*) to obtain **4** (11 mg), **5** (8 mg), **6** (16 mg) and **7** (13 mg). Compound **4** further separated by using Semi-Preparative HPLC (CH_3CN/H_2O, 15 : 85 *v*/*v*, ULTRON ES-OVM column) to obtain **4a (**3 mg**)** and **4b** (4 mg).

2.4. Spectroscopic data

Decumbic acid A (**1**): white powder; $[\alpha]^{24}_D$ +85 (*c* 1.2, MeOH); IR (KBr) ν_{max} 1785, 1730 and 1210cm^{-1}; ^{1}H and ^{13}C NMR: see Table 1; HRESIMS *m/z* 185.0808 $[M - H]^-$ (calcd for $C_9H_{13}O_4$, 185.0814).

Decumbic acid B (**2**): white powder; $[\alpha]^{24}_D$ +21 (*c* 0.8, MeOH); IR (KBr) ν_{max} 1782, 1722 and 1216cm^{-1}; ^{1}H and ^{13}C NMR: see Table 1; HRESIMS *m/z* 185.0816 $[M - H]^-$ (calcd for $C_9H_{13}O_4$, 185.0814).

(-)-Decumbic acid (**3a**): white powder; $[\alpha]^{24}_D$ -43 (*c* 1.5, MeOH); IR (KBr) ν_{max}

3422, 2962, 2878, 1741, 1665 and 1218cm^{-1}; ^{1}H and ^{13}C NMR: see Table 1; HRESIMS *m/z* 183.0646 [M - H]$^{-}$ (calcd for $C_9H_{11}O_4$, 183.0657).

(-)-Dendrolactone (**4a**): white powder; $[\alpha]^{24}_{D}$ -108 (*c* 1.0, MeOH); IR (KBr) ν_{max} 3425, 1778, 1616 and 1508cm^{-1}; ^{1}H and ^{13}C NMR: see Table 1; HRESIMS *m/z* 279.0878 [M - H]$^{-}$ (calcd for $C_{14}H_{15}O_6$, 279.0869).

(+)-Dendrolactone (**4b**): white powder; $[\alpha]^{24}_{D}$ +110 (*c* 0.8, MeOH); IR (KBr) ν_{max} 3424, 1778, 1617 and 1508cm^{-1}; ^{1}H and ^{13}C NMR: see Table 1; HRESIMS *m/z* 279.0872 [M - H]$^{-}$ (calcd for $C_{14}H_{15}O_6$, 279.0869).

Crystal data for decumbic acid (raceme **3**): $C_9H_{12}O_4$ M = 184.19; monoclinic, space group $P_{21/c}$; a = 4.93786 (12) Å, b = 19.0807 (4) Å, c = 10.3251 (2) Å, $\alpha = \gamma$ = 90.00° β = 101.961 (2)°, V = 951.69 (4) Å^3, Z = 4, T = 293 (2) K, D_c = 1.285 g/cm^3, μ(Cu Kα) = 0.854 mm^{-1}, F (000) = 392, 9323 reflections measured, 1517 independent reflections (R_{int} = 0.0287). The final R_1 values were 0.0365 [$I > 2\sigma(I)$]. The final wR_2 (F^2) values were 0.1028 [$I > 2\sigma(I)$]. The final R_1 values were 0.0407 (all data). The final wR_2 (F^2) values were 0.1075 (all data).

Crystal data for decumbic acid (raceme **4**): $C_{14}H_{16}O_6$ M = 280.27; monoclinic, space group $P_{21/c}$; a = 10.6264 (4) Å, b = 7.1627 (3) Å, c = 17.1423 (6) Å, $\alpha = \gamma$ = 90.00° β = 98.792 (3)°, V = 1289.44 (8) Å^3, Z = 4, T = 293 (2) K, D_c = 1.444 g/cm^3, μ(Cu Kα) =0.960 mm^{-1}, F (000) = 592, 12642 reflections measured, 1907 independent reflections (R_{int} = 0.0333). The final R_1 values were 0.0397 [$I > 2\sigma(I)$]. The final wR_2 (F^2) values were 0.1070 [$I > 2\sigma(I)$]. The final R_1 values were 0.0488 (all data). The final wR_2 (F^2) values were 0.1178 (all data).

Crystal X-ray diffraction data was collected on a Bruker APEX DUO diffractometer with Cu Kα radiation (λ = 1.54184 Å). The structure was solved by direct methods (SHELXS-97) and refined using full-matrix least-squares difference Fourier techniques. Carbon and oxygen atoms were refined anisotropically. Hydrogen atoms were either refined freely with isotropic displacement parameters or positioned with an idealized geometry and refined riding on their parent C atoms. Crystals suitable for X-ray diffraction (racemes **3** and **4**) were obtained by slow evaporation of a solution in MeOH/$CHCl_3$ (10:1). Crystallographic data (excluding structure factors) for racemes **3** and **4** have been deposited with the Cambridge Crystallographic Data

Centre: CCDC reference number 1499064 and 1499065, respectively. This data can be obtained, free of charge, from the Cambridge Crystallographic Data Centre via http://www.ccdc.cam.ac. uk/data_request /cif.

Cells. HeLa, MCF-7 and A549 were provided by College of Pharmacy, Hebei University and maintained in DMEM medium (Gibco) containing 5% fetal bovine serum (Gibco) at 37°C in air with 5% CO_2.

MTT Assays. Cytotoxic activity was evaluated by the MTT method, as described previously[12]. In the MTT assay, drug stock solutions were prepared in DMSO (stored at –70°C). Cells were harvested at the exponential growth phase and seeded in flat-bottomed 96-well microtiter plates. The cell volume in each well was 180 μL, containing 10^4 cells per well. The plates were incubated overnight in a 5% humidified CO_2 incubator at 37°C. The compounds were then added to each well at various concentrations using a constant volume of 20 μL. After 48 h incubation at 37°C in 5% CO_2, 50 μL of MTT (1 mg/mL of PBS) was added to each well and again incubated at 37°C for 4 h. Following incubation, the medium was replaced by DMSO (150 μL) to dissolve the formazan crystals in each well. The plates were then read at 570 nm wavelength in an enzyme-labeled detector (Elx800, BioTek Instruments, Inc.). IC_{50} values of the compounds in different cell lines were determined based on the dose response curve. Epirubicin was used as a positive control.

Fungal Strains. *Alternaria brassicicola*, *Phytophthora parasitica var. nicotianae*, *Colletotrichum capsici*, *Bipolaris oryzae*, *Diaporthe medusaea nitschke*, *Ceratocystis paradoxa moreau*, *Exserohilum turcicum*, *Pestallozzia theae* and *Alternaria A. citri* were grown on potato dextrose agar.

Antifungal Bioassay. The antifungal bioassays were conducted following the National Center for Clinical Laboratory Standards (NCCLS) recommendations. Targeted microbes (three to four colonies) were prepared from broth culture (28°C for 48 h), and the final spore suspensions of fungi were 10^4 mycelial fragments/mL. Test compounds (1 mg/mL as the stock solution in DMSO and serial dilutions) were transferred to a 96-well clear plate in triplicate, and the suspension of the test

organisms was added to each well achieving a final volume of 200 μL. The plates were stored in controlled-environment cabinets from 4 to 14 days, depending on the assay, and mycelial growth was then assessed. After incubation, the absorbance at 492 nm was measured with a microplate reader. The MIC was defined as the lowest test concentration that completely inhibited the growth of the test organisms. Prochloraz was used as a positive control.

3. Results and discussion

Compound **1** was obtained as white powder, with the molecular formula $C_9H_{14}O_4$ from HRESIMS data combined with ^{1}H and ^{13}C NMR spectroscopic data (Table 1). The ^{1}H NMR data revealed two methyl groups at δ_H 0.95 (t, J = 7.2 Hz) and 1.32 (d, J = 7.2 Hz), one oxygenated methine signal at δ_H 4.71 (td, J = 8.4, 3.2 Hz), two methine signals at δ_H 3.21 (dd, J = 9.2, 8.4 Hz) and 3.03 (m). The ^{13}C NMR and 135 DEPT data showed 9 resonances, including two carboxyl carbons (δ_C 177.8 and 174.6), one oxygenated methine carbon (δ_C 77.4), two sp^3 methine carbons (δ_C 51.8 and 36.7), two methylene carbons (δ_C 33.28 and 19.1) and two methyl carbons (δ_C 14.6 and 13.8). These data closely resembled those of known compound **3** except for the absence of two olefinic carbons (δ_C 147.1 and 139.9, C-2 and C-3) and the presence of two sp^3 methine carbons (δ_C 51.8 and 36.7) in **1**. The 2D structure of **1** was thus constructed. It was corroborated by the ^{1}H-^{1}H COSY and HMBC correlations. The ^{1}H-^{1}H COSY correlations of H-8 to H-2, H-2 to H-3, H-3 to H-4, H-4 to H-5, H-5 to H-6 and H-6 to H-7 indicated the presence of the C-8/C-2/C-3/C-4/C-5/C-6/C-7 fragment in **1** (Fig. 2). The HMBC correlations from H-2/H-4 to C-9 indicated that C-9 was linked to C-3. The HMBC correlations from H-8/H-3 to C-1 indicated that C-2 was linked to C-1. The HMBC correlations from H-4 to C-1 indicated the presence of the five-membered lactone ring. The relative configuration of **1** was assigned on the basis of the NOESY experiment. The NOESY correlations of 8-Me /H-3, 8-Me /H-4 and H-2/H-5 indicated that 8-Me, H-3 and H-4 should be placed on the same face; H-2 and the propyl group should be placed on the other face (Fig 3). Compound **1** has the (2*R*,3*R*,4*R*)-form based upon the positive optical rotation of **1** and the reported similar compounds in literatures [13,14]. Thus, the absolute configuration of **1** was defined as (2*R*,3*R*,4*R*) (Fig. 4). Thus, compound **1** was identified as a new butyrolactone derivative. And we named compound **1** as decumbic acid A.

Compound **2** was also obtained as white powder, with the molecular formula $C_9H_{14}O_4$ from HRESIMS data combined with ^1H and ^{13}C NMR spectroscopic data (Table 1). The 1D NMR data were very similar to those of **1** except for the small shifting of the chemical shift at C-2, C-3, C-4 and C-5 in **2** (Table 1). Detailed analysis of 2D NMR (HSQC, ^1H-^1H COSY and HMBC) spectra confirmed that compound **2** has the same 2D structure with **1**. The relative configuration of **2** was assigned on the basis of the NOESY experiment. The NOESY correlations of 8-Me/H-3, H-3/H-5 and H-2/H-4 indicated that 8-Me and the propyl group should be placed on the same face; the carboxyl group, H-2 and H-4 should be placed on the other face (Fig 3). Compound **2** has the (2*S*,3*S*,4*R*)-form based upon the positive of **2** and the reported similar compounds in literatures [13,14]. Therefore, decumbic acid B (**2)** was identified as a new butyrolactone derivative.

Raceme **3** was obtained as colorless crystals. The 1D NMR data were same as the decumbic acid (known compound 3b) [8]. However, **3** was determined as a raceme by the crystal space group (Fig. 4). Furthermore, subsequent HPLC separation on an ULTRON ES-OVM column (150 × 10 mm, 5 μm) was successful in resolving two stereoisomes (**3a** and **3b**), which were opposite in terms of the specific rotations (**3a**, -43 and **3b**, +44). **3b** was a known compound with the (4*R*)-form ($[\alpha]^{24}_D$ +44). On the contrary,, the absolute configuration of **3a** was defined as (4*S*) ($[\alpha]^{24}_D$ -43) and we named compound **3a** as (-)-decumbic acid.

Raceme **4** was also obtained as colorless crystals. The 1D NMR data were very similar to those of caruilignan C [15] except for the small shifting of the chemical shift at C-2−6. Detailed analysis of 2D NMR (HSQC and HMBC) spectra confirmed that compound **4** has the same 2D structure with caruilignan C. The relative configuration of **4** was assigned on the basis of the crystal space group (Fig. 5). Compound **4** as a raceme was also determined by the crystal space group. (2*S*,5*S*,6*R*)-Form and (2*R*,5*R*,6*S*)-form were found in the crystal space. Furthermore, subsequent HPLC separation on an ULTRON ES-OVM column (150 × 10 mm, 5 μm) was also successful in resolving two stereoisomes (**4a** and **4b**), which were opposite

in terms of the specific rotations (**4a,** -108 and **4b,** +110). The (2*R*,5*R*,6*S*)-form for **4b** was based upon the optical rotation. It's $[\alpha]^{24}_{D}$ (+110) was very close to the reported similar compound (+)-(7*S*,8*R*,8'*R*)-acuminatolide (+100.1) in literatures [16]. On the contrary, compound 4a has the (2*S*,5*S*,6*R*)-Form. Thus, the absolute configuration of **4a** and **4b** was defined as (2*S*,5*S*,6*R*) and (2*R*,5*R*,6*S*), respectively. We named compounds **4a** and **4b** as (-)- and (+)-dendrolactone, respectively.

The structures of known compounds **3b** and **5–7** were identified by comparison of their spectroscopic data with those in the literature.

Cytotoxic Activities. All compounds were tested for cytotoxic activities against HeLa, MCF-7 and A549 cells (table 2). Compounds **1**–**7** showed moderate inhibitory effects on HeLa, MCF-7 and A549 cells with IC_{50} values ranging from 15.3 to 30.0 μM. The IC_{50} values of other compounds higher than 40 μM were regarded as inactive.

[a] Epirubicin was used as a positive control.

Antifungal Activities. All compounds were evaluated against the phytopathogenic fungi and the results are shown in Table 3.

Acknowledgments

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Supplementary data

Supplementary matrial 1

Supplementary matrial 2

Supplementary figures

References

[1] Jiangsu New Medical College. *Dictionary of Chinese Medicines*; Shanghai Scientific and Technologic Press: Shanghai, China, 1986; pp 586–590.

[2] Kim, J. K. *Illustrated Natural Drugs Encyclopedia*; Nam Sand Dang: Seoul, 1989; Vol. II, p 181.

[3] H. K. Wang, T. F. Zhao, C. T. Che. Dendrobine and 3-Hydroxy-2-oxodendrobine from *Dendrobium nobile*. J. Nat. Prod. 48 (1985) 796–801.

[4] X. Zhang, J. K. Xu, J. Wang, H. Kurihara, S. Kitanaka, X. S. Yao. Bioactive Bibenzyl derivatives and fuorenones from *Dendrobium nobile*. J. Nat. Prod. 70 (2007) 24–48.

[5] W. Zhao, Q. Ye, X. Tan, H. Jiang, X. Li, K. Chen, A. D. Kinghorn. Three new sesquiterpene glycosides from *Dendrobium nobile* with immunomodulatory activity. J. Nat. Prod. 64 (2001) 1196–1200.

[6] Y. H. Lee, J. D. Park, N. I. Baek, S. I. Kim, B. Z. Ahn. In vitro and in vivo antitumoral phenanthrenes from the aerial parts of *Dendrobium nobile*. Planta Med. 61 (1995) 178–180.

[7] X. M. Zhou, C. J. Zheng, L. S. Gan, G. Y. Chen, X. P. Zhang, X. P. Song, G. N. Li, C. G. Sun. Bioactive phenanthrene and bibenzyl derivatives from the stems of *Dendrobium nobile*. J. Nat. Prod. 79 (2016) 1791–1797.

[8] G. He, H. Matsuura, T. Yoshihara. Isolation of an α-methylene-γ-butyrolactone derivative, a toxin from the plant pathogen *Lasiodiplodia theobromae*. Phytochemistry. 65 (2004) 683–688.

[9] X. Y. Gao, N. L. Wang, X. S. Yao. Chemical constituents of *Pholidota yunnanensis* rolfe. Journal of Shenyang Pharmaceutical University. 23 (2006) 205–208.

[10] R. C. Lin, A. L. Skaltsounis, E. Seguin, F. Tillequin, M. Koch. Phenolic constituents of *Selaginella doederleinii*. Planta Med. 60 (1994) 168–170.

[11] A. S. Feliciano, M. Medarde, J. L. Lopez, J. M. M. D. Corral. Two new cinnamyl isovalerate derivatives from *Juniperus thurifera* leaves. J. Nat. Prod. 49 (1986)

677–679.

[12] D. A. Scudiero, R. H. Shoemaker, K. D. Paull, A. Monks, S. Tierney, T. H. Nofziger, M. J. Currens, D. Seniff, M. R. Boyd. Evaluation of a soluble tetrazolium/formazan assay for cell growth and drug sensitivity in culture using human and other tumor cell lines. Cancer Res. 48 (1988) 4827−4833.

[13] S. Drioli, F. Felluga, C. Forzato, P. Nitti, G. Pitacco, E. Valentin. Synthesis of (+)- and (-)-phaseolinic acid by combination of enzymatic hydrolysis and chemical transformations with revision of the absolute configuration of the natural product. J. Org. Chem. 63 (1998) 2385−2388.

[14] S. Hajra, A. Karmakar, A. K. Giri, S. Hazra. Concise syntheses of (+)- and (-)-methylenolactocins and phaseolinic acids. Tetrahedron Lett. 49 (2008) 3625−3627.

[15] C. M. Ma, N. Nakamura, B. S. Min, M. Hattori. Triterpenes and lignans from *Artemisia caruifolia* and their cytotoxic effects on Meth-A and LLCtumor cell lines. Chem. Pharm. Bull. 49 (2001) 183−187.

[16] R. Saladino, C. Fiani, C. Crestini, D. S. Argyropoulos, S. Marini, M. Coletta. An efficient and stereoselective dearylation of asarinin and sesamin tetrahydrofurofuran lignans to acuminatolide by methyltrioxorhenium/H_2O_2 and UHP systems. J. Nat. Prod. 70 (2007) 39−24.

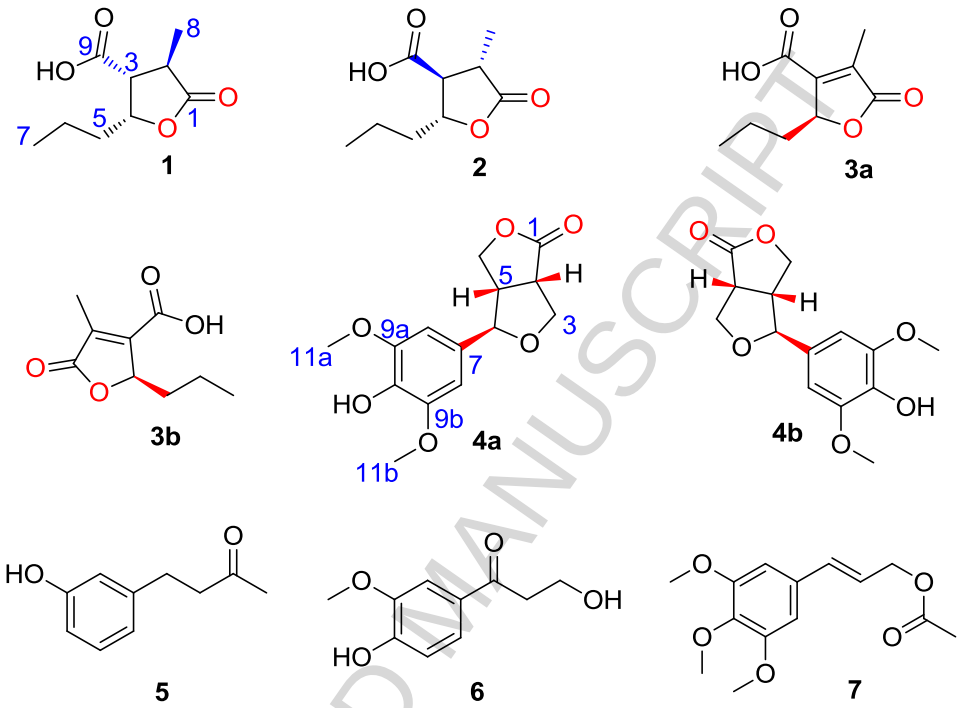

Fig 1. Structures of compounds **1**–**7** isolated from *D. nobile*.

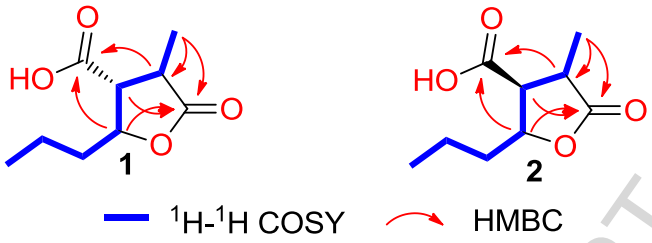

Fig. 2. Key ^{1}H-^{1}H-COSY and HMBC correlations (H→C) of **1** and **2**

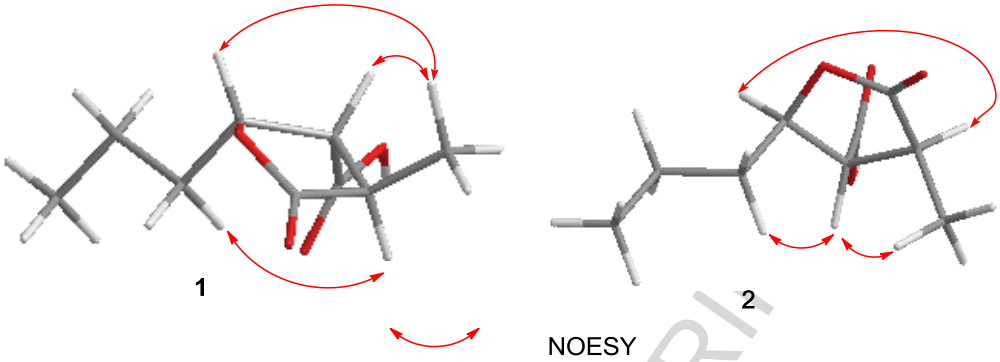

Fig. 3. Key NOESY correlations of **1** and **2**

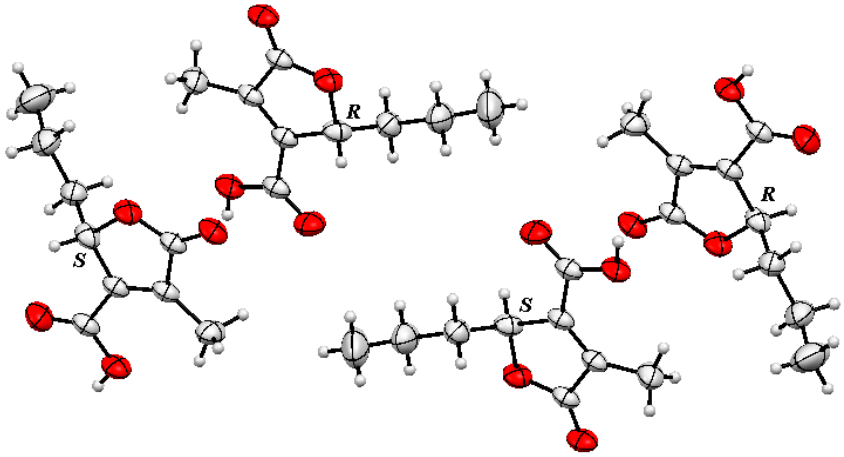

Fig. 4. The crystal space group of the raceme **3**

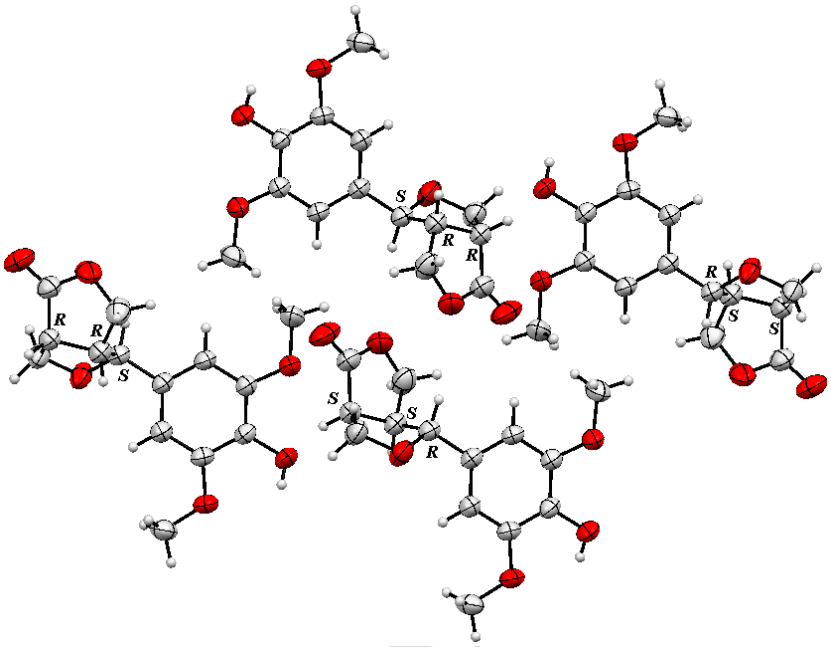

Fig. 5. The crystal space group of the raceme **4**

Table 1. NMR Data for compounds 1, 2, 3a, 3b, 4a and 4b

position	**1** (in $CDCl_3$)		**2** (in $CDCl_3$)		**3a/3b** (in $CDCl_3$)		**4a/4b** (in $CDCl_3$)	
	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
1	-	177.8	-	177.7	-	172.9	-	178.2
2	3.03 (m)	36.7	3.00 (m)	39.9	-	139.9	3.46 (m)	46.2
3	3.21 (dd, 9.2, 8.4)	51.8	2.70 (dd, 11.2, 10.0)	54.0	-	147.1	4.39 (dd, 9.2, 8.8, H-3*β*) 4.20 (dd, 9.2, 2.8, H-3*α*)	70.2
4	4.71 (td, 8.4, 3.2)	77.4	4.51 (td, 8.8, 4.0)	79.8	5.13 (dd, 8.0, 2.4)	81.4	4.51 (dd, 10.0, 6.8, H-4*β*) 4.34 (dd, 10.0, 2.0, H-4*α*)	69.9
5	1.59 (m)	33.2	1.71/1.82 (m)	36.9	1.59/2.11 (m)	34.9	3.11 (m)	48.7
6	1.43/1.62 (m)	19.1	1.45/1.55 (m)	18.7	1.44 (m)	18.3	4.59 (d, 6.8)	86.4
7	0.95 (t, 7.2)	13.8	0.97 (t, 7.2)	13.7	0.95 (t, 7.2)	13.8	-	130.0
8 (8a/8b)	1.32 (d, 7.2)	14.6	1.33 (d, 7.2)	14.5	2.24 (s)	11.2	6.57 (s)	102.9
9 (9a/9b)	-	174.6	-	174.7	-	166.5	-	147.5
10	-	-	-	-	-	-	-	135.0
11 (11a/11b)	-	-	-	-	-	-	3.91 (s)	56.6

Table 2. The cytotoxic Activities of Isolated Compounds

Compounds	IC_{50} (μM)		
	HeLa	MCF-7	A549
1	25.3	24.3	25.8
2	18.2	15.3	19.5
3a	20.2	18.9	20.8
3b	18.8	17.5	19.2
4a	24.7	21.7	23.4
4b	21.5	28.2	19.3
5	22.0	18.6	20.5
6	21.5	21.9	23.6
7	22.3	30.0	28.2
Epirubicin [a]	0.5	0.8	1.0

Table 3. The Antifungal Activity of Isolated Compounds

Cpds	MIC (μg/mL)								
	A. brassicicola	*P. parasitica var. nicotianae*	*C. capsici*	*B. oryzae*	*D.medusaea Nitschke*	*C. paradoxa Moreau*	*E. turcicum*	*P. theae*	*A. citri*
1	>400	100	>400	>400	50	50	>400	50	>400
2	50	>400	>400	>400	100	>400	50	>400	>400
3a	>400	100	50	>400	>400	>400	>400	>400	50
3b	>400	100	50	>400	>400	>400	>400	>400	50
4a	>400	>400	>400	>400	100	>400	>400	50	>400
4b	>400	>400	>400	>400	100	>400	>400	50	>400
5	50	100	50	100	>400	>400	>400	>400	50
6	50	>400	25	100	>400	50	25	>400	>400
7	>400	50	>400	100	100	>400	50	50	>400
Prochloraz[a]	12.5	50	12.5	50	50	25	12.5	25	25

[a] Prochloraz was used as a positive control.

Graphical abstract