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**Two new tetrahydrofuran derivatives from the fungus *Mucor* spp.
SNB-VECD11D isolated from the Chrysomelidae *Acalymma
bivittula***

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Abstract

Two new tetrahydrofuran derivatives, mucorinic acids A (**1**) and B (**2**) as well as the three known secondary metabolites dehydroabietic acid (**3**), Δ 8-dihydroabietic acid (**4**) and 8-pimarenic acid (**5**) were isolated from a solid culture of the fungus *Mucor* spp. isolated on insect *Acalymma bivittula*. The structure of these compounds was elucidated by analysis of NMR and MS spectroscopic data. Compounds were tested in antimicrobial and insecticidal assays. Dehydroabietic acid exhibited moderate larvicidal activity on *Aedes aegypti* larvae with 65% mortality at 10 ppm. Both new compounds **1** and **2** showed interesting antibacterial activity on *Staphylococcus aureus* and methicillin resistant *S. aureus* with MIC values in the 8–16 μ g/ml.

Keywords :

Mucor spp.; fungus; insecticide; antibacterial; *Aedes aegypti*

The search of insecticidal and antimicrobial molecules represents a global challenge for public health. On one hand, vector-borne diseases account for over 17% of all infectious diseases.¹ In particular, mosquito transmit a number of diseases such as malaria (*Anopheles* sp.), filariasis (*Culex* sp.), dengue and more recently Zika virus (*Aedes* sp.), causing millions of deaths every year.² On the other hand, most antimicrobial drugs suffer from major drawbacks such as emergence of resistant/multiresistant strains and toxicity. Hence, it is critical to discover new, more efficient and safer insecticides and antimicrobials.

Entomopathogenic microorganisms are natural enemies of insects. They produce a large diversity of secondary metabolites during the process of infection and proliferation, including insecticides and antimicrobials.³ As such, entomopathogens are considered as an underrated source of novel bioactive compounds of pharmaceuticals and ecological interests.^{4,5}

Based on this dual potential, we have undertaken to search for new insecticides and antimicrobials from a collection of 57 microorganisms isolated from the cuticle of alive, yet infected insect species. All strains were cultivated and extracted with ethyl acetate. Extracts were screened on human pathogenic microorganisms and on *Aedes aegypti* larvae. The fungus *Mucor* spp. exhibited promising larvicidal and antibacterial properties. *Mucor* fungus belongs to the class of Zygomycota. They were often described as plant pathogens, but can also be deadly pathogens for insects.

Results and discussion.

The fungus cultivated on potato dextrose agar and identified as *Mucor* spp. SNB-VECD11D proved very active on *Ae. aegypti* larvae (laboratory reference strain Paea) with mortality rate of 77% after 24h of exposure at a dose of 100 µg/ml. It was also active on *Staphylococcus aureus* and methicillin-resistant *S. aureus* (MRSA) with minimal inhibitory concentrations (MIC) of 64 µg/ml and 16 µg/ml, respectively. The chemical study of *Mucor* spp. extract led to the isolation of two new tetrahydrofurans derivatives **1** and **2**, dehydroabietic acid ⁶ (**3**), Δ8-dihydroabietic acid (**4**) and 8-pimarenic acid (**5**) (Figure 1).

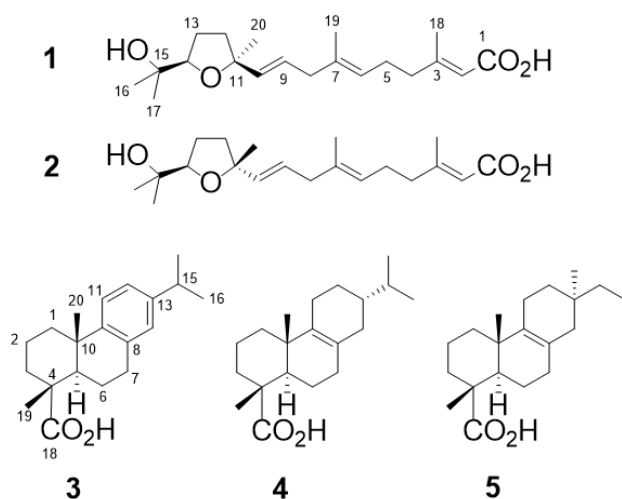


Fig. 1. Structure of compounds 1–5

Compound **1** was isolated as pale yellow oil. Its molecular formula was C₂₀H₃₂O₄ as determined by HRESITOFMS analysis, which showed a pseudomolecular ion at m/z 337.2394 [M+H]⁺ (calcd for C₂₀H₃₃O₄, 337.2373), corresponding to five degrees of unsaturation.

The ¹H NMR spectrum of **1** suggested the presence of five methyl groups at δ_H 1.15, 1.16, 1.29, 1.61 and 2.07, and five methines corresponding to δ_H 5.68, 5.62, 5.60, 5.17 and 3.84 (Table 1). The ¹³C, HSQC and HMBC NMR data allowed the identification of five quaternary

carbons at δ_C 173.6, 154.5, 135.7, 84.0 and 72.5. Three double bonds and a carbonyl group were detected in HSQC. These accounted for four out of the five unsaturations. The sequence of ^1H - ^1H COSY correlations H-10/ H-9/H-8/H-7/H-6/H-5 and H-4/H-3/H-2 combined with the HMBC correlations H-10/C-8, C-9 ; H-8/C-7, C-9 and C-10 ; H-6/C-4 and C-5 ; H-4/C-2 and C-3 established a farnesyl-like polyene side chain from C-10 to C-2. Methyl substituents 18 and 19 were connected to C-3 and C-7, respectively. HMBC correlations of protons H-18 to C-1 placed the carboxylic group at the end of the chain (Fig. 2). The *E* configuration of the double bonds can be established based on NOESY correlations H-8/H-6, H-10 and H-2/H-4 (Fig. 3). The tetrahydrofuran moiety was determined based on COSY correlations between H-12 (δ_H 1.84/1.94), H-13 (δ_H 1.78/1.87) and H-14 (δ_H 3.84) combined with HMBC correlations of protons H-12 to C-11 (δ_C 84.0), C-13 (δ_C 39.0) and C-14 (δ_C 86.5). The farnesyl side chain was linked to the tetrahydrofuran in C-11 as established by the correlation H-10/C-11. The correlation between methyl groups 16 (δ_H 1.15) and 17 (δ_H 1.16) with C-14 and C-15 defined a 2-hydroxypropan-2-yl subunit linked to C-14.

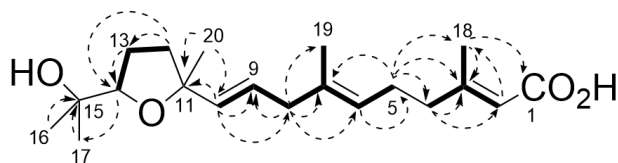


Fig. 2. Key ^1H - ^1H COSY (bold line) and HMBC (dashed arrows) correlations of **1**.

The molecular formula and the chemical shifts in C-11 (δ_C 84.0) and C-14 (δ_C 86.5) suggested these two carbons were linked by an oxygen therefore forming a tetrahydrofuran ring. This was confirmed by a NOE correlation between H-14 and H-20, also indicating that H-14 and methyl 20 are on the same side of the ring (Fig. 3). Overall compound **1** was the (2*E*,6*E*,9*E*)-

5-(2-hydroxypropan-2-yl-2-methyltetrahydrofuran-2-yl)-3,7-dimethyldeca-2,6,9-trienoic acid and was named mucorinic acid A.

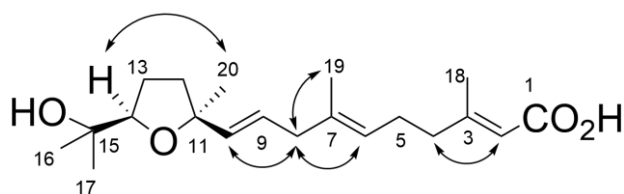


Fig. 3. Key NOESY correlations of **1**.

Compound **2** was obtained as pale yellow oil. Its molecular formula was determined as $C_{20}H_{32}O_4$ based on HRESITOFMS experiment (m/z 337.2348 $[M+H]^+$). This compound has five degrees of unsaturation and the 1H , ^{13}C and HSQC NMR data revealed that it was an analogue of compound **1**. The main difference was observed in the ROESY analysis. In compound **2**, H-9 and H-10 correlate with H-14 therefore confirming the relative configuration proposed in figure 1. This compound was named mucorinic acid B.

Table 1. NMR Spectroscopic Data (CD_3OD) for compounds **1** and **2**

Pos.	1		2	
	$\delta_C^{a,c}$, type	δ_H^b (J in Hz)	δ_C^a , type	δ_H^b (J in Hz)
1	173.6, C		173.0, C	
2	120.74, CH	5.68, m	119.7, CH	5.68, m
3	154.5, C		156.4, C	
4	41.44, CH_2	2.14, m	41.5, CH_2	2.15, m
5	27.1, CH_2	2.20, m	27.1, CH_2	2.21, m
6	125.3, CH	5.17, t (6.9)	125.1, CH	5.17, t (6.9)
7	135.7, C		135.8, C	
8	43.5, CH_2	2.68, d (6.5)	43.3, CH_2	2.68, d (6.3)
9	126.8, CH	5.60, m	126.6, CH	5.56, m
10	138.8, CH	5.62, m	138.1, CH	5.51, m
11	84.0, C		84.1, C	
12	27.6, CH_2	a: 1.84, m b: 1.94, m	27.4, CH_2	a: 1.84, m b: 1.88, m
13	39.0, CH_2	a: 1.78, m b: 1.87, m	38.4, CH_2	a: 1.67, m b: 1.86, m

14	86.5, CH	3.84, m	86.6, CH	3.78, m
15	72.5, C		72.5, C	
16	25.8, CH ₃	1.15, br s	25.6, CH ₃	1.15, s
17	25.8, CH ₃	1.16, br s	25.7, CH ₃	1.16, s
18	18.5, CH ₃	2.07, s	18.6, CH ₃	2.09, s
19	16.0, CH ₃	1.61, s	16.0, CH ₃	1.61, s
20	26.3, CH ₃	1.29, s	27.4, CH ₃	1.30, s

^aData recorded at 150MHz. ^bData recorded at 600MHz.

^cCarbon shift were obtained based on HSQC and HMBC data.

Isolated compounds from *Mucor* spp. strain were evaluated for their antimicrobial activities against several human pathogens and for their larvicidal activities against *Ae. aegypti* larvae. Dehydroabietic acid (**3**) exhibited larvicidal activity on *Ae. aegypti* whereas the other four compounds exhibited moderate antimicrobial activity but no antifungal activity (Table 2).

Table 2. Antimicrobial and larvicidal activity (at 10 ppm) of compounds **1–5**

Compound	Larvicidal activity (% mortality)	MIC (µg/ml)			
		<i>C. albicans</i>	<i>S. aureus</i>	MRSA	<i>T. rubrum</i>
1	2.0	> 256	16	16	> 256
2	2.1	> 256	8	16	> 256
3	65.0	> 256	128	64	64
4	6.2	> 256	16	16	> 256
5	10.0	> 256	16	16	> 256
Pos. Ctrl. ^a		4	0.2	0.6	4

^a Postitive control : oxacillin for *S. aureus* ; fluconazole for *C. albicans* and *T. rubrum* ; vancomycine for MRSA.

In conclusion this letter reports the first description of two new antibacterial tetrahydrofuran derivatives mucorinic acids A and B. The same fungus also produces abietane or pimarane diterpenoids **3–5**. Compound **3** known as a conifer biomarker has been discovered later from fungi and cyanobacteria.^{7,8} *Mucor* spp. SNB-VECD11D is another fungus able to produce abietanes and pimaranes.

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A. Supporting information

Supplementary data (NMR data of compounds **1**, **2**, **3**, **4** and **5** as well as MS data of **1** and **2**) associated with this article are available online.

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