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New acorane sesquiterpenes isolated from the endophytic fungi *Colletotrichum gloeosporioides* SNB-GSS07

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Abstract:

Looking for new anti-glycation compounds, four acorane sesquiterpenes together with seven known molecules were isolated from *Colletotrichum gloeosporioides*, an endophytic fungus of *Sabicea cinerea*. Their structures were established based on mass spectrometry and NMR, together with circular dichroism for their absolute configurations.

Endophytes are a group of microorganisms, mostly bacteria and fungi, which reside inside all plant species.¹ These microorganisms contribute to the defense and resilience of their host.² They can be viewed as a rich source of new compounds with original biological activities.³

Colletotrichum gloeosporioides is a fungus from the Glomerellaceae family. It is distributed worldwide, and has been described as a phytopathogene or an endophyte.^{3,4} This fungal species can be responsible for the anthracnose disease in tropical fruit-bearing plants like mango and papaya, but has also been isolated as an endophyte of many healthy plants. A large diversity of secondary metabolites such as gloeosporone,⁴ ferricrocin,⁵ colletotric acid,⁶ 2-phenylethyl-1H-indol-3-yl-acetate,⁷ 2-(4-hydroxyphenyl)acetic acid,⁷ 2-(2-hydroxyphenyl)acetic acid,⁷ or 4-hydroxybenzamide have been isolated from *C. gloeosporioides* strains.⁷

Our group has been investigating for a few years non-cytotoxic fungal extracts looking for new anti-glycation agents. The ethyl acetate extract of *C. gloeosporioides* SNB-GSS07, which had been isolated from the leaves of *Sabicea cinerea*,⁸ exhibited interesting activity. Thorough chemical investigation of this extract, obtained from a solid culture of *C. gloeosporioides* strain, led to the isolation of four acoranes (**1-4**) that had not been described before in the literature.

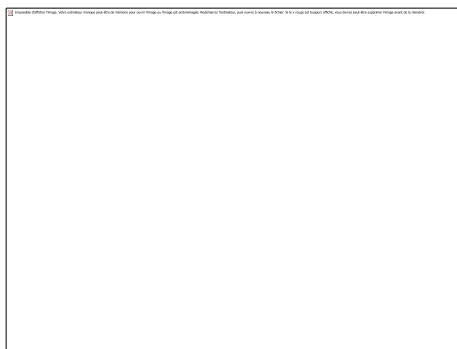


Figure 1. Structure of compounds **1-4**

Compound **1** HRESIMS analysis indicated a molecular formula of $C_{15}H_{24}O_3$ (m/z 253.1808 for $[M+H]^+$). The planar structure of **1** was deduced from interpretation of 1D and 2D NMR experiments (Table 1, Table S1). 1H and ^{13}C NMR spectra, along with HSQC experiment indicated the presence of four methyls (δ_C 15.2, δ_H 1.71; δ_C 17.6, δ_H 0.92; δ_C 22.5, δ_H 0.97; δ_C 24.7, δ_H 1.06), two methylenes (δ_C 39.1, δ_H 1.62/2.13 ; δ_C 51.7, δ_H 2.08/2.95), four methines among which two were oxygenated (δ_C 30.2, δ_H 1.87; δ_C 69.7, δ_H 4.79; δ_C 79.0, δ_H 3.99; δ_C 152.7, δ_H 6.61), and three quaternary carbons (one aliphatic carbon at δ_C 57.8, one olefinic carbon at δ_C 135.2, one α,β -unsaturated carbonyl moiety at δ_C 202.1).

The 1H - 1H COSY spectrum showed two coupling systems leading to the description of two partial structures (Figure 2). On one side the sequence of correlations H14, H4, H3, H2, H1, H11, H12/H13, allowed building the C1-C4 partial structure, substituted in C1 by an isopropyl group, in C3 by a hydroxyl, and in C4 by a methyl. On the other side, the H10 oxygenated methyne correlated with the olefinic proton H9 that, in turn, correlated with H15. In HMBC (Figure 2), protons H14, H4, H2, H1, H11 correlated to C5, and so did protons H10, H9 and H6b. Furthermore, H6a and H6b correlated to the ketone C7.

Overall it was possible to identify a C5 spiro ring system with a cyclopentane ring on one side and a 4-hydroxy-2-methyl-cyclohex-2-en-1-one moiety on the other side.

Compound **1** was an acorane type sesquiterpenoid. Its planar structure was identical to that of 3,10-dihydroxyacoronene.⁹ Eventually, it was possible to find out differences in the relative configuration as established by analysis of the ROESY spectrum (Figure 3). First, the relative configuration at C1, C5 and C10 can be determined based on the sequence of correlations H1–H6b–H10–H11/12. The model presented here was built based on Feng's 3,10-dihydroxyacoronene crystallographic data.¹⁰ It shows H6b and H10 in pseudo-axial position with the cyclohexenone ring in a half chair conformation. This observation is consistent with the experimental ROE measurements. The correlation between H6a and H4 proved the relative configuration at C4, and the sequence of ROE correlations H14–H3–H2b–H11 confirmed the relative configuration at C-3.

The absolute configuration was obtained by comparison of the CD spectrum with the one recorded by Feng.¹¹ Both spectra exhibited a negative Cotton effect at 320 nm (Figure S2). This wavelength corresponds to the $n\text{-}\pi^*$ transition of the $\alpha\text{-}\beta$ -unsaturated ketone. In the 7-oxo-acoranes series, the sign of the Cotton effect depends on the absolute configuration of the spiro carbon, which was therefore identical in 3,10-dihydroxyacoronene and in **1**. The octant rule would also provide a negative sign for the Cotton effect.¹¹ These data demonstrated that the absolute configuration of compound **1** was 1*R*,3*S*,4*S*,5*S*,10*R*. Compound **1** was new to Science and was named after acorenone (3*S*,10*R*)-1,4-*epi*-3,10-dihydroxyacorenone.¹²

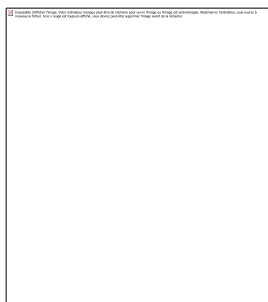


Figure 2. Key COSY (bold lines) and HMBC (dashed arrows) correlations of compounds **1**

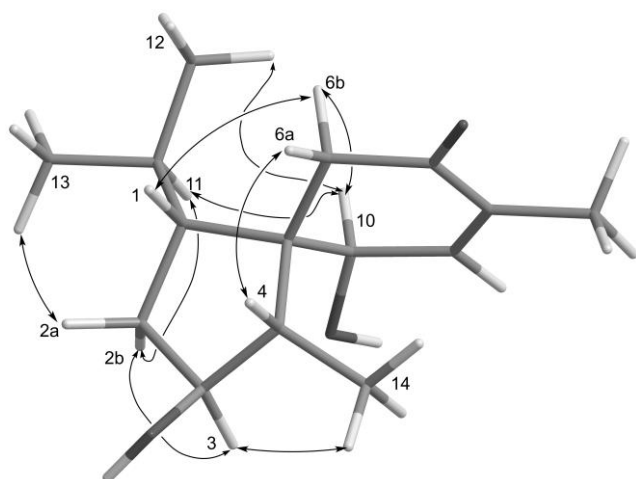


Figure 3. Key ROE correlations of compound **1**

Table 1. ^1H and ^{13}C NMR spectroscopic data of compounds **1**, **2**, **3** and **4** in CD_3OD

Pos.	1			2			3			4		
	δ_{C}	δ_{H} , m (J in Hz)		δ_{C}	δ_{H} , m (J in Hz)		δ_{C}	δ_{H} , m (J in Hz)		δ_{C}	δ_{H} , m (J in Hz)	
1	55.9, CH	1.55, br. q (9.0)		55.6, CH	1.61, ddd (10.5, 8.7, 6.1)		57.7, CH	2.13, m		61.9, CH	1.87, m	
2	39.1, CH_2	a: 1.62, ddd (13.1, 9.5, 2.2)		36.3, CH_2	a: 1.663, m		34.5, CH_2	a: 1.54, ddd (13.2, 8.9, 3.7)		25.3, CH_2	1.70, m	
		b: 2.13, br. q (11.6)			b: 1.86, ddd (13.5, 10.7, 8.7)			b: 2.05, br. td (13.2, 11.1, 8.5)				
3	79.0, CH	3.99, br. td (8.6, 2.3)		77.5, CH	3.72, br. td (8.5, 3.2)		78.2, CH	4.11, ddd (8.0, 3.5)		32.7, CH_2	a: 1.63, m	
											b: 1.81, m	
4	57.0, CH	1.56, br. quint (6.8)		55.9, CH	1.50, quint (7.2)		56.6, CH	1.55, br. quint (7.1)		48.9, CH	1.77, m	
5	57.8, C			50.2, C			59.4, C			58.4, C		
6	51.7, CH_2	a: 2.08, d (15.9)		50.9, CH_2	a: 2.31, d (16.5)		45.9, CH_2	a: 2.11, dd (13.3, 7.5)		45.7, CH_2	a: 2.06, dd (13.7, 8.8)	
		b: 2.95, d (15.9)			b: 2.69, d (16.5)			b: 2.14, dd (13.3, 7.5)			b: 2.10, dd (13.7, 6.2)	
7	202.1, C			202.7, C			68.1, CH	4.61, br. t (7.5)		68.2, CH	4.59, brt (7.6)	
8	135.2, C			136.5, C			166.1, C			165.2, C		
9	152.7, CH	6.61, br. s		147.0, CH	6.77, br. t (4.3)		130.3, CH	5.78, br. s		130.5, CH	5.75, quint (1.4)	
10	69.7, CH	4.79, br. s		28.2, CH_2	a: 2.15, br. d (19.6)		204.1, C			205.0, C		
					b: 2.39, br. d (19.6)							
11	30.2, CH	1.87, oct (6.7)		30.0, CH	1.669, m		30.1, CH	1.86, m		30.4, CH	1.89, m	
12	24.7, CH_3	1.06, d (6.8)		21.6, CH_3	0.88, d (6.7)		18.8, CH_3	0.66, d (6.6)		25.1, CH_3	0.89, d (6.7)	
13	22.5, CH_3	0.97, d (6.8)		24.6, CH_3	0.96, d (6.7)		25.2, CH_3	0.90, d (6.9)		19.3, CH_3	0.70, d (6.5)	
14	17.6, CH_3	0.92, d (7.1)		15.2, CH_3	0.88, d (6.7)		12.3, CH_3	0.92, d (6.9)		15.0, CH_3	0.88, d (6.7)	
15	15.2, CH_3	1.71, br. s		15.6, CH_3	1.72, br. s		20.3, CH_3	2.03, br. s		20.4, CH_3	2.02, brt (1.2)	

The molecular formula of compound **2** was determined to be $C_{15}H_{24}O_2$ by HRESIMS (m/z 237.1845 for $[M+H]^+$), indicating one fewer oxygen atom compared to **1**. The planar structure of **2** was deduced by interpretation of 1D and 2D NMR experiments (Table 1, Table S2). NMR data were rather similar to those of **1**, except for the obvious presence of a methylene with non-equivalent protons (δ_C 28.2; δ_H 2.15/2.39) instead of an oxygenated methyne (δ_C 69.7; δ_H 4.79) in position 10. Detailed NMR data table and a figure of correlations are given in supplementary material (Table S2, Figure S1). The relative configuration of **2** was determined based on the NOESY experiment. Briefly, the coupling of H6a with H1 and H4 along with the coupling of H6b with H13 allowed us to define the relative configuration of carbons C1, C4 and C5 and the relative position of H6a and H6b. Then, the correlations between H10a and both H14 and H3, and the coupling of H10b with H11, H12 and H13 confirmed the established relative configuration and allowed us to establish the relative configuration of carbon C3. Overall, the relative configuration was the same as for compound **1**. As regards absolute configuration, the CD spectrum again showed a negative Cotton effect at 320 nm which indicated that the absolute configuration at C-5 was identical to that of 3,10-dihydroxyacoronene and **1** (Figure S2). Therefore, due to the Cahn-Ingold-Prelog priority rules, the absolute configuration of **2** was 1*R*,3*S*,4*S*,5*R*. This new acorane sesquiterpene was named (3*S*)-1,4-*epi*-3-hydroxyacorenone.

Compound **3** HRESIMS analysis indicated a molecular formula of $C_{15}H_{24}O_3$ (m/z 253.1803 for $[M+H]^+$), as for compound **1**. The planar structure of **3** was deduced from interpretation of 1D and 2D NMR experiments (Table 1, Table S3). The C1–C4 partial structure was identical to that of **1** (Figure 4). However, on the other side of the molecule, the methylene protons H6 correlated to an oxygenated methine H7, which in

turn correlated with H15. In HMBC, protons H2a, H4, H11 and H14 correlated to C5, and so did protons H6, H7 and H9. Moreover, H1, H4, H6 and H15 all correlated to ketone in C10, and H9, H6, H7 correlated to the oxygenated carbon C7. Overall, it was found that **3** was an isomer of compound **1** with a carbonyl group on C10 and a hydroxyl on C7.

The relative configuration of **3** was determined by analysis of the ROESY spectrum (Figure 4). The relative configuration of the stereogenic centers C1, C4 and C5 was established based on correlations between H6a and H4 on one side on the cyclohexenone, and H6b and H12 on the other side. The configuration at C7 was defined by the correlations of H7 with H6b and H11. Lastly, NOE coupling between H2b and both H3 and H14, along with a coupling between H14 and H3, indicated that these protons were on the same side of the cyclopentane ring, therefore confirming the relative stereochemistry at C3 as represented in Figure 5. The relative stereochemistry was identical to that of compounds **1** and **2**, and it is highly unlikely that the absolute configuration was the opposite based on biosynthetic considerations. Hence, the absolute configuration of **3** was 1*S*,3*S*,4*S*,5*S*,7*R*. Compound **3** was given the trivial name (3*S*,7*R*)-1,4-*epi*-3,7-dihydroxyacoren-10-one.

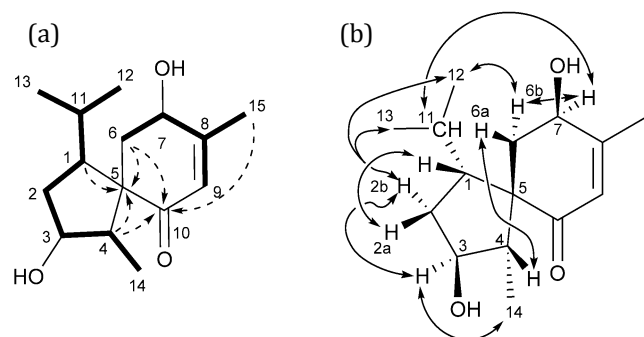


Figure 4. (a) Key COSY (bold lines) and HMBC (dashed arrows) correlations of compound **3** ; (b) Key ROESY correlations of compound **3**.

The molecular formula of compound **4** was determined to be C₁₅H₂₄O₂ by HRESIMS (m/z 237.1836 for [M+H]⁺), indicating one fewer hydroxyl group compared to **3**. The planar structure of **4** was determined using 1D and 2D NMR analysis, and by comparison to data of compound **3** (Table 1, Table S4). The main difference was in the cyclopentane moiety, with one methylene (δ_C 32.7, δ_H 1.81/1.63) instead of a methine. The COSY and HMBC correlations confirmed the loss of the hydroxyl group in position C3 (see also Figure S1). The relative configuration of **4** was determined by the analysis of the NOESY spectrum. Briefly, couplings between H4 and H6b, and between H6a and H1/H12 defined the stereocenters C1, C4 and C5. Then correlations of H7 with H6a and H11 established the relative configuration in C7. The relative configuration was identical to those of compounds **1-3**, and biosynthetic considerations would provide the absolute stereochemistry as 1*R*,4*R*,5*R*,7*R*. This new compound was named (7*R*)-1,4-*epi*-7-hydroxyacoren-10-one.

Other compounds isolated in the ethyl acetate extract of SNB-GSS07 were Sumiki's acid (**5**),¹³ acetyl Sumiki's acid (**6**),¹⁴ convolvulopyrone (**7**),¹⁵ *p*-hydroxybenzaldehyde (**8**),¹⁶ 4-hydroxyphenylacetic acid (**9**),¹⁷ indole-3-carboxylic acid (**10**),¹⁸ and indole-3-carboxaldehyde (**11**).¹⁹ Compounds **5-11** were identified by comparison of their spectral data with those reported in the literature.

In conclusion, four new acorane sesquiterpenes were isolated from the endophytic fungi *Colletotrichum gloeosporioides* SNB-GSS07. Their structures and absolute configurations were determined by mass spectrometry, NMR and circular dichroism. To our knowledge, this is the first time that acorane type sesquiterpenes have been isolated

from endophytic sources. Literature indicates isolations of these kinds of structures from various plants, but never from associated microorganisms.

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