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## Verbenanone, an octahydro-5H-chromen-5-one from a Hawaiian-Plant Associated Fungus FT431

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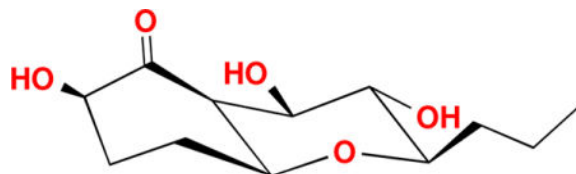
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### Abstract

A new secondary metabolite verbenanone (**1**) with a unique (4a*S*,8a*S*)-octahydro-5H-chromen-5-one moiety has been obtained from the endophytic fungus FT431, which was isolated from the native Hawaiian plant *Verbena* sp. The structure of compound **1** was characterized based on NMR and MS spectroscopic analysis. The absolute configuration (AC) of compound **1** was determined by Mosher acids. Compound **1** was tested against A2780 and A2780cisR, but it was inactive.

### Graphical abstract



### Keywords

Hawaii; *Verbena*; endophytic fungi

Fungi have been a great source of many biologically active compounds for drug development.<sup>1,2</sup> For examples, the famous antibiotic penicillin G was obtained from many *Penicillium* strains; the immunosuppressant medication cyclosporine was isolated from

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Notes: The authors declare no competing financial interest.

### Supplementary Material

Supplementary data (NMR spectra of the new compound) associated with this article can be found, in the online version, at [www.sciencedirect.com](http://www.sciencedirect.com).

*Tolypocladium inflatum* Gams;<sup>3</sup> the cholesterol lowering agent mevastatin (ML-236B) is produced by the fungus *Penicillium citrinum*.<sup>4</sup> Endophytic fungi, which live symbiotically within cells of higher plants, produce structurally diverse and biologically active compounds.<sup>5–10</sup> During an ongoing search for new and bioactive compounds from Hawaiian endophytic fungi, about 5,000 semi-pure fractions from fungal culture extracts were screened against cisplatin-sensitive A2780 (A2780, human ovarian cancer) and cisplatin-resistant A2780 (A2780cisR, Stat3 abnormally activated) cell lines. The results showed that one semi-pure fraction produced by an endophytic fungus FT431 was active against A2780 and A2780cisR at 20  $\mu\text{g}/\text{mL}$ . From one FT431 fraction, compound **1** (Figure 1) was obtained, which was inactive against A2780 and A2780cisR.

Compound **1**<sup>11</sup> was isolated as a colorless solid. Its molecular formula was determined to be  $\text{C}_{12}\text{H}_{20}\text{O}_5$  by HR-ESIMS ( $m/z$  261.1336, calcd for  $[\text{M} + \text{H}_2\text{O} - \text{H}]^-$  261.1338), with three degrees of unsaturation. The IR spectrum showed the existence of a ketone carbonyl ( $1723\text{ cm}^{-1}$ ) and hydroxyl ( $3335\text{ cm}^{-1}$ ) groups. A detailed analysis of  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra (Table 1) demonstrated the presence of one methyl signal, four methylenes, six methines including five oxygenated, and a carbonyl carbon with no hydrogen attached. In the  $^1\text{H}$ - $^1\text{H}$  COSY spectrum of **1**, only one spin system was readily identified, from H-2 all the way to H<sub>3</sub>-12, CH-CH<sub>2</sub>-CH<sub>2</sub>-CH-CH-CH-CH-CH-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>3</sub> (Figure 2). Since there was no double bond in **1**, the three degrees of unsaturation must be due to the ketone and two rings in the molecule. In the HMBC spectrum of **1**, H-5 showed correlation to C-9 and H-9 correlated to C-5 (Figure 2), indicating that a tetrahydro-2H-pyran ring was formed between C-5 and C-9 since the  $^{13}\text{C}$  chemical shifts of C-5 and C-9 were  $\delta_{\text{C}}$  79.4 and 81.9 ppm, respectively (Table 1). The  $^{13}\text{C}$  chemical shifts of C-2, C-7 and C-8 were  $\delta_{\text{C}}$  76.5, 75.6 and 72.7 ppm, respectively, so these three positions must be oxygenated. In the HMBC spectrum, H-2, H-3, H-5, H-6 and H-7 correlated to C-1 (212.4), indicating a carbonyl carbon between C-2 and C-6. H-7 showed a weak HMBC correlation to C-2 ( $\delta_{\text{C}}$  76.5), due to a four-bond w-shaped coupling<sup>12</sup>. Hence, the planar structure of **1** was determined.

The large coupling constants ( $J = 9.3\text{ Hz}$ ) of H-8 with H-7 and H-9 meant that H-7, H-8 and H-9 must be in axial positions. The ROESY correlations between H-5 and H-7, H-5 and H-9, H-7 and H-9 indicated that H-5 was at the same side of the ring as H-7 and H-9, and it was also in an axial position. H-6 must be in an equatorial position due to its small coupling constants (br s), and it was also at the same side of ring with H-5 and H-7. The ROESY correlation between H-2 and H-6 suggested a  $\beta$ - orientation of the hydroxyl group at 1-position. Hence the relative configuration of compound **1** was determined as shown in Figure 1.

To determine the absolute configuration, compound **1** was converted to the two Mosher esters **1a** and **1b** with (*S*)- and (*R*)-MTPA-Cl.<sup>11,13</sup> The resulting esters **1a** and **1b** (Figure 3) were subjected to NMR analysis. The chemical shift differences  $\delta^{SR}$  were significant (Figure 3), which made it possible to conclude that **1** had the *R*-configuration at C-7. Based on the relative configuration determined above, the configuration at C-2, C-5, C-6, C-7, C-8 and C-9 was determined to be *R*, *S*, *S*, *R*, *S*, and *R*, respectively.

In order to validate the proposed assignment, and intrigued by the chemical shift of C-6 (more deshielded than expected for any regular methine), we undertook quantum chemical calculations of NMR shifts. This approach represents a useful and simple strategy for the elucidation of complex organic molecules,<sup>14</sup> and has been extensively employed in the recent past to settle structural issues of a wide variety of natural products.<sup>14,15</sup> As shown in Table 2, the chemical shifts of compound **1** computed at the PCM/mPW1PW91/6-31+G\*\*//PCM/B3LYP/6-31G\* level of theory (using methanol as solvent) nicely matched our experimental findings. The overall agreement was high, with CMAE (corrected mean average error, defined as  $\sum_n |\delta_{sc} - \delta_{exp}|/n$ ) values of 1.3 ppm (<sup>13</sup>C) and 0.09 ppm (<sup>1</sup>H), and CMaxErr (corrected maximum error, defined as  $\max |\delta_{sc} - \delta_{exp}|$ ) of only 2.7 ppm (<sup>13</sup>C) and 0.19 ppm (<sup>1</sup>H).

Despite the experimental NMR observations discussed above provided a strong evidence to support the stereochemistry suggested for **1**, we also computed the NMR shifts for all the remaining 31 possible diastereoisomers of **1** to strengthen the confidence in our assignment (Isomers 2–32, see the SI). To our delight, we noticed that in such cases the agreement between experimental and calculated was not as good as in the case of isomer **1** (with all the configurations indicated for **1**). For instance, the CMAE values of isomers 2–32 ranged 1.5–3.7 ppm (<sup>13</sup>C) and 0.09–0.29 ppm (<sup>1</sup>H), higher than those computed for isomer **1** (1.3 ppm and 0.09 ppm, respectively), showing higher CMaxErr values as well (3.0–12.2 ppm for carbon data, 0.21–0.99 ppm for proton data). With this data in hand, we finally computed the DP4+ probability,<sup>16</sup> among the preferred strategies to assess the most likely structure when only one set of experimental data is available.<sup>14,16</sup> As expected, the DP4+ values strongly suggested isomer **1** as the correct candidate in high confidence (>99.9%).

During the analysis of the MS (–ve) spectrum of compound **1**, we were puzzled by the ion peaks at 245 and 261. We proposed that the ketone at 1-position of **1** was hydrated when the molecule was pushed into the spectrometer. Loss of an OH from the hydrated **1** would generate *m/z* 245 (Figure 4).

Biogenetically, **1** could be derived from an unsaturated long chain molecule (**i**). A 6 $\pi$  electrocyclization event would furnish **ii**, that after the etherification process indicated in Figure 5 would entail the core chromene-like structure present in **iii**. Further hydrogenation, hydroxylation, and oxidation of **iii** could generate compound **1** (Figure 5).

While octahydro-2*H*-chromene derivatives are very common, small molecule octahydro-2*H*-chromenes with hydroxyl groups at 1- and 2-positions are unusual. For examples, compounds **2–4**<sup>17,18</sup> (Figure 6) are hydroxylated octahydro-2*H*-chromenes, but they are synthesized compounds. Hydroxylated (4*aS*,8*aS*)-octahydro-5*H*-chromen-5-one<sup>19</sup> analogs are rarer, even for organically synthetic compounds.

Compound **1** was tested against A2780 and A2780cisR, but it was inactive.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

## Acknowledgments

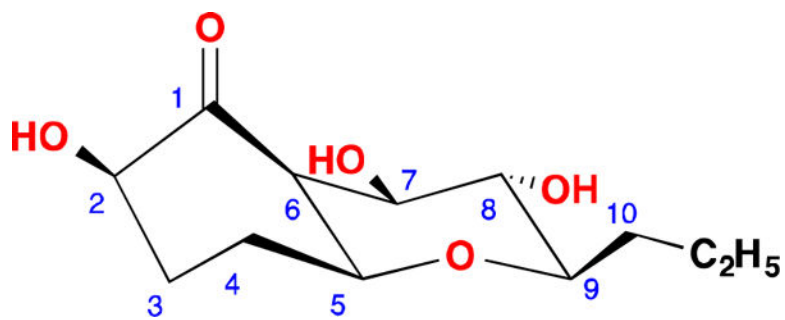
This work was financially supported mainly by a start-up funding from University of Hawaii Cancer Center, University of Hawaii at Hilo, and the Victoria S. and Bradley L. Geist Foundation (15ADVC-74420) (to SC) and the National Institutes of Health (NIH)/National Cancer Institute (NCI) Grant CA128865 (JT).

## References and notes

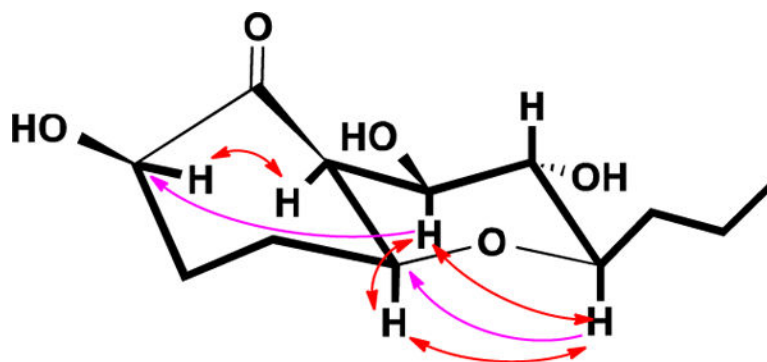
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- a) General experimental procedures: Optical rotation was measured with a Rudolph Research Analytical AutoPol IV Automatic Polarimeter. UV and IR spectra were obtained with Shimadzu UV-1800 spectrophotometer and Thermo scientific Nicolet iS50FT-IR spectrometer, respectively. NMR spectra including 1D and 2D experiments were recorded in methanol- $d_4$  on a Bruker 400 MHz NMR; CD spectrum was recorded on Jasco J-815 circular dichroism spectrophotometer in methanol. HPLC was carried out on Thermo scientific Ultimate 3000 LC system, and all solvents were HPLC grade. Column chromatography used Diaion HP-20 (Sigma). b) Isolation and identification of fungal strain: The fungal strain was isolated on PDA medium from a healthy leaf of Hawaiian indigenous plant, *Verbena* sp., which was collected in Lyon Botanic Garden in 2014. The strain FT431 was identified as *Peyronellaea* sp. based on the analysis of the DNA sequence of the nuclear ribosomal internal transcribed spacer, which has been deposited in GenBank with the accession no. KY971272. A voucher specimen was deposited at Daniel K. Inouye College of Pharmacy, University of Hawaii at Hilo, USA (accession no. FT431). c) Cultivation: The fungus was grown under static condition at room temperature for 30 days in 1 L conical flask containing the liquid medium (300 mL/flask) composed of mannitol (20 g/L), sucrose (20 g/L), monosodium glutamate (5 g/L),  $\text{KH}_2\text{PO}_4$  (0.5 g/L),  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$  (0.3 g/L), yeast extract (3 g/L), corn steep liquor (2 g/L) at pH 6.5. d) Isolation of compound 1: The fermented whole broth (6 L) was filtered through filter paper to separate the supernatant from the mycelia. The supernatant solution was passed through Diaion HP-20 eluted with MeOH– $\text{H}_2\text{O}$  (10%, 30%, 50%, 70% and 90%) to afford five fractions (Fr. A–E). Fr. B (258.60 mg) was further fractionated by preparative HPLC (Phenyl Hexyl column,  $100.0 \times 21.2$  mm; 10 mL/min; with 0.1% formic acid in mobile phases) eluted with 20–50% MeOH– $\text{H}_2\text{O}$  in 30 min to get 30 sub-fractions (B1–B30). B18 (7.20 mg) was purified by semi-preparative HPLC (C18 column,  $250.0 \times 10.0$  mm; 3 mL/min; with 0.1% formic acid in 13%  $\text{CH}_3\text{CN}/\text{H}_2\text{O}$ ) to obtain compound 1 (2.75 mg,  $t_R$  33.4 min). (1) white powder;  $[\alpha] +9.4$  (c 0.32, MeOH); UV (MeOH)  $\lambda_{\text{max}}$  203, 268 nm; IR (film)  $\nu_{\text{max}}$  3335, 2955, 2870, 2360, 2342, 1723, 1653, 1605, 1559, 1457, 1437, 1397, 1375, 1358, 1253  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (400 MHz,  $\text{CD}_3\text{OD}$ ) and  $^{13}\text{C}$  NMR (100 MHz,  $\text{CD}_3\text{OD}$ ): see Table 1; HRESIMS  $m/z$  261.1336  $[\text{M}+\text{H}_2\text{O}-\text{H}]^-$  (calcd for  $\text{C}_{12}\text{H}_{21}\text{O}_6$ , 261.1338). e) Reaction with Mosher reagents: Acylation of compound 1 (0.7 mg, 2.87  $\mu\text{M}$  each) with *S*(+) and *R*(–)- $\alpha$ -methoxy- $\alpha$ -(trifluoromethyl) phenyl acetyl chloride (MTPA-Cl) $^{13}$  (21.43  $\mu\text{M}$  each) yielded 2'-MTPA esters 1a (1.26 mg, 1.87  $\mu\text{M}$ ) and 1b (1.32 mg,

- 1.95  $\mu\text{M}$ ), respectively (Fig. 3). The  $^1\text{H}$  NMR signals of the MTPA esters were assigned on the basis of their COSY spectra, and the  $\delta_{\text{H}}(\text{S-R})$  values were then calculated (Fig. 3).<sup>13</sup>
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- Verbenanone with an uncommon structure has not been reported before.
- Absolute configuration of verbenanone was determined.
- NMR calculation was utilized to determine the stereochemistry of verbenanone.
- The biosynthesis of verbenanone has been proposed.

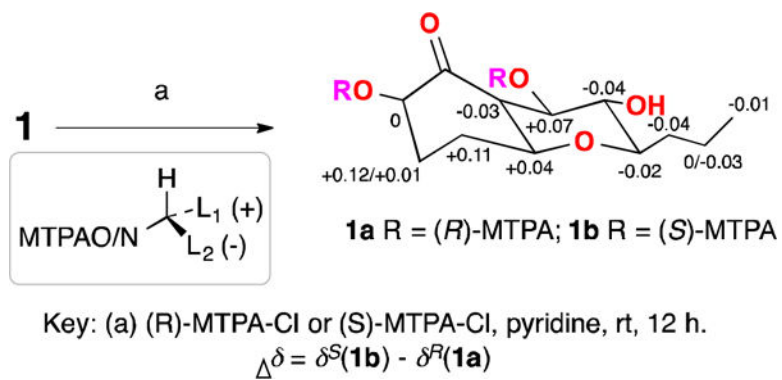


**Figure 1.**  
Structure of compound 1

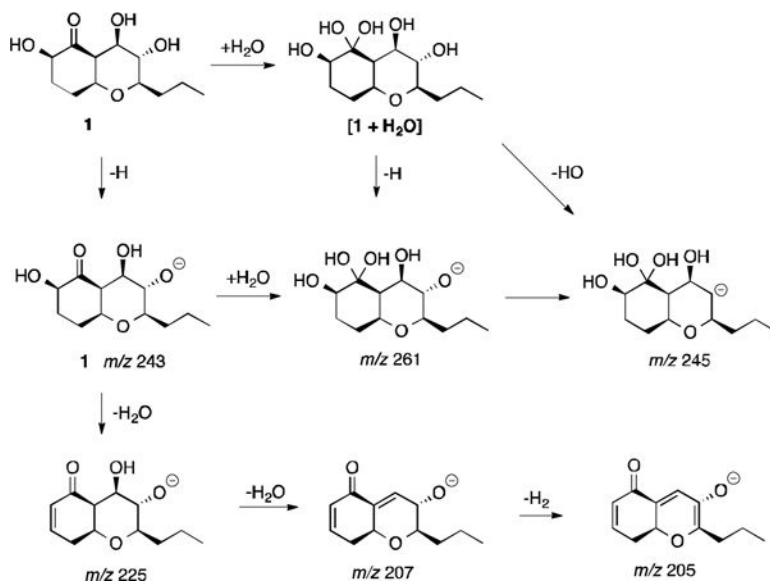


**Figure 2.**  
COSY (Bold), key HMBC (Single headed) and ROESY (Double headed) correlations of **1**

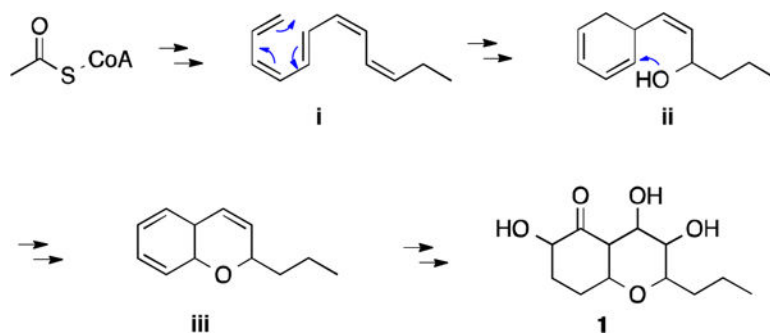




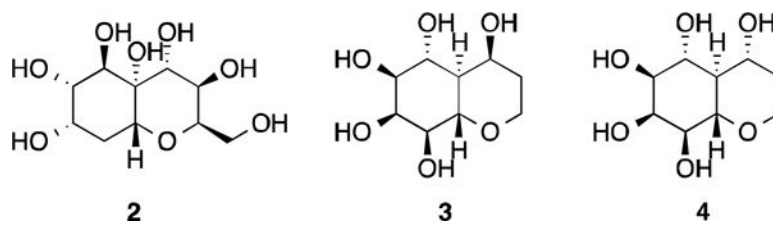
**Figure 3.**  
 Reactions of compound **1** with Mosher esters



**Figure 4.**  
Proposed fragmentation mechanism of compound **1**



**Figure 5.**  
Proposed biosynthetic pathway for compound **1**



**Figure 6.**  
Some multiple hydroxylated octahydro-2*H*-chromenes

**Table 1**NMR Spectroscopic Data for **1** in MeOH-*d*<sub>4</sub>

no.	<b>1</b>		ROESY correlations
	$\delta_{\text{H}}, J$ (Hz) <sup>a</sup>	$\delta_{\text{C}}$ <sup>b</sup>	
1		212.4	
2	4.24, dd, 12.0, 6.7	76.5	6
3	1.90, m 2.14, m	32.8	
4	2.03, m	29.2	11a, 11b, 12
5	3.97, d, 2.6 m	79.4	4, 6, 7, 9
6	3.15, br s	55.2	2, 4, 5, 7
7	3.49, dd, 9.3, 5.0	75.6	5, 6, 9
8	3.63, dd, 9.3, 9.3	72.7	
9	3.08, ddd, 11.7, 9.3, 2.6	81.9	5, 7, 10a, 10b
10	1.38, m 1.78, m	35.3	
11	1.35, m 1.49, m	19.7	11a-3
12	0.92, t, 7.2	14.6	4, 11a, 11b

<sup>a</sup>Spectra recorded at 400 MHz.<sup>b</sup>Spectra recorded at 100 MHz. Data based on <sup>1</sup>H, <sup>13</sup>C, HSQC, and HMBC experiments.

**Table 2**Calculated  $^1\text{H}$  and  $^{13}\text{C}$  NMR shifts of **1**

no.	<b>1</b>			
	Exp $\delta_{\text{H}}$	Calc $\delta_{\text{H}}$	Exp $\delta_{\text{C}}$	Calc $\delta_{\text{C}}$
1			212.4	212.0
2	4.24	4.32	76.5	73.7
3	1.90	2.03		
	2.14	2.25	32.8	32.3
4	2.03	2.02	29.2	28.6
5	3.97	4.02	79.4	77.2
6	3.15	3.00	55.2	51.7
7	3.49	3.38	75.6	73.8
8	3.63	3.43	72.7	70.4
9	3.08	3.21	81.9	77.6
10	1.38	1.32		
	1.78	1.79	35.3	34.1
11	1.35	1.36		
	1.49	1.58	19.7	20.3
12	0.92	0.90	14.6	14.1