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Verbenanone, an octahydro-5*H*-chromen-5-one from a Hawaiian-plant associated fungus FT431

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ABSTRACT

Verbenanone (**1**), a new secondary metabolite with a unique (4a*S*,8a*S*)-octahydro-5*H*-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.

Fungi have been a great source of many biologically active compounds for drug development.[1,2] For examples, the famous antibiotic penicillin G was obtained from many *Penicillium* strains; the immunosuppressant medication cyclosporine was isolated from *Tolypocladium inflatum Gams*[3]; the cholesterol lowering agent mevastatin (ML-236B) is produced by the fungus *Penicillium citrinum*.[4] Endophytic fungi, which live symbiotically within cells of higher plants, produce structurally diverse and biologically active compounds.[5–10] During an ongoing search for new and bioactive compounds from Hawaiian endophytic fungi, about 5000 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 A2780-cisR at 20 μg/mL. From one FT431 fraction, compound **1** (Fig. 1) was obtained, which was inactive against A2780 and A2780cisR.

Compound **1**[11] was isolated as a colorless solid. Its molecular formula was determined to be $C_{12}H_{20}O_5$ by HR-ESIMS (*m*/*z* 261.1336, calcd for $[M+H_2O-H]^-$ 261.1338), with three degrees of unsaturation. The IR spectrum showed the existence of a ketone carbonyl (1723 cm^{-1}) and hydroxyl (3335 cm^{-1}) groups. A detailed analysis of ^1H and ^{13}C NMR spectra (Table 1) demonstrated the

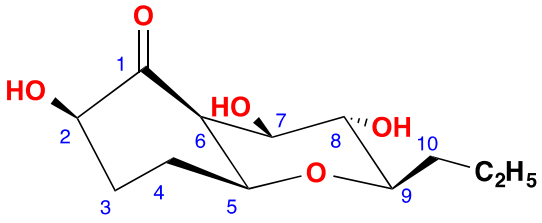

Fig. 1. Structure of compound **1**.

presence of one methyl signal, four methylenes, six methines including five oxygenated, and a carbonyl carbon with no hydrogen attached. In the ^1H-^1H COSY spectrum of **1**, only one spin system was readily identified, from H-2 all the way to H_3-12, CH–CH_2–CH_2–CH–CH–CH–CH–CH–CH_2–CH_2–CH_3 (Fig. 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 (Fig. 2), indicating that a tetrahydro-2H-pyran ring was formed between C-5 and C-9 since the ^{13}C chemical shifts of C-5 and C-9 were δ_C 79.4 and 81.9 ppm, respectively (Table 1). The ^{13}C chemical shifts of C-2, C-7 and C-8 were δ_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 (δ_C 212.4), indicating a carbonyl carbon between C-2 and C-6. H-7 showed a weak HMBC correlation to C-2 (δ_C 76.5), due to a

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Table 1
NMR Spectroscopic Data for **1** in MeOH-d_4.

No.	**1**		
	δ_H, J (Hz)[a]	δ_C[b]	ROESY correlations
1		212.4	
2	4.24, dd, 12.0, 6.7	76.5	6
3	1.90, m	32.8	
	2.14, m		
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	35.3	
	1.78, m		
11	1.35, m	19.7	11a-3
	1.49, m		
12	0.92, t, 7.2	14.6	4, 11a, 11b

[a] Spectra recorded at 400 MHz.
[b] Spectra recorded at 100 MHz. Data based on ^{1}H, ^{13}C, HSQC, and HMBC experiments.

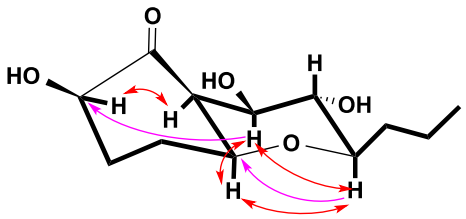
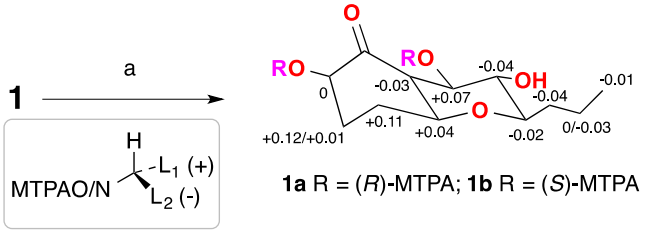


Fig. 2. COSY (Bold), key HMBC (Single headed) and ROESY (Double headed) correlations of **1**.

four-bond w-shaped coupling.[12] Hence, the planar structure of **1** was determined.

The large coupling constants (J = 9.3 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 β-orientation of the hydroxyl group at 1-position. Hence the relative configuration of compound **1** was determined as shown in Fig. 1.

To determine the absolute configuration, compound **1** was converted to the two Mosher esters **1a** and **1b** with (*S*)- and (*R*)-MTPA-Cl.[11,13] The resulting esters **1a** and **1b** (Fig. 3) were subjected to NMR analysis. The chemical shift differences $\Delta\delta^{SR}$ were significant (Fig. 3), which made it possible to conclude that **1** had the *R*-configuration at C-7. Based on the relative configuration determined



Key: (a) (R)-MTPA-Cl or (S)-MTPA-Cl, pyridine, rt, 12 h.
$\Delta\delta = \delta^S$(**1b**) - δ^R(**1a**)

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

Table 2
Calculated ^{1}H and ^{13}C NMR shifts of **1**.

No.	**1**			
	Exp δ_H	Calc δ_H	Exp δ_C	Calc δ_C
1			212.4	212.0
2	4.24	4.32	76.5	73.7
3	1.90	2.03	32.8	
	2.14	2.25		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	35.3	
	1.78	1.79		34.1
11	1.35	1.36	19.7	
	1.49	1.58		20.3
12	0.92	0.90	14.6	14.1

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[14], and has been extensively employed in the recent past to settle structural issues of a wide variety of natural products.[14,15] 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 $\Sigma_n|\delta_{sc} - \delta_{exp}|/n$) values of 1.3 ppm (^{13}C) and 0.09 ppm (^{1}H), and CMaxErr (corrected maximum error, defined as $\max|\delta_{sc} - \delta_{exp}|$) of only 2.7 ppm (^{13}C) and 0.19 ppm (^{1}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 (^{13}C) and 0.09–0.29 ppm (^{1}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,[16] among the preferred strategies to assess the most likely structure when only-one set of experimental data is available[14,16]. 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 (Fig. 4).

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

While octahydro-2*H*-chromene derivatives are very common, small molecule octahydro-2*H*-chromenes with hydroxyl groups