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

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Abstract

A new secondary metabolite verbenanone (1) with a unique (4aS,8aS)-octahydro-5H-chromen-5-one moiety has been obtained from the endophytic fungus FT431, which was isolated from the native Hawaiian plant \textit{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; \textit{Verbena}; endophytic fungi

Fungi have been a great source of many biologically active compounds for drug development.\textsuperscript{1,2} For examples, the famous antibiotic penicillin G was obtained from many \textit{Penicillium} strains; the immunosuppressant medication cyclosporine was isolated from
To determine the absolute configuration, compound 1 was converted to the two Mosher esters 1a and 1b with (S)- and (R)-MTPA-Cl. The resulting esters 1a and 1b (Figure 3) were subjected to NMR analysis. The chemical shift differences $\Delta \delta_{SR}$ 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,\textsuperscript{14} and has been extensively employed in the recent past to settle structural issues of a wide variety of natural products.\textsuperscript{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 $\sum_n |\delta_{sc} - \delta_{exp}| / n$) 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,\textsuperscript{16} among the preferred strategies to assess the most likely structure when only one set of experimental data is available.\textsuperscript{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 (Figure 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 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-2H-chromene derivatives are very common, small molecule octahydro-2H-chromenes with hydroxyl groups at 1- and 2-positions are unusual. For examples, compounds 2–4\textsuperscript{17,18} (Figure 6) are hydroxylated octahydro-2H-chromenes, but they are synthesized compounds. Hydroxylated (4aS,8aS)-octahydro-5H-chromen-5-one\textsuperscript{19} analogs are rarer, even for organically synthetic compounds.

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

\section*{Supplementary Material}

Refer to Web version on PubMed Central for supplementary material.
Acknowledgments

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References and notes

1. Schueffler A, Anke T. Nat Prod Rep. 2014; 31:1425–1448. [PubMed: 25122538]
2. Evidente A, Kornienko A, Cimmino A, Andolfi A, Lefranc F, Mathieu V, Kiss R. Nat Prod Rep. 2014; 31:617–627. [PubMed: 24651312]
3. Cyclosporin: Petcher TJ, Weber HP, Rüegger A. Helv Chim Acta. 1976; 59:1480–1489. [PubMed: 931750]
4. Mevastatin: Endo A, Kuroda M, Tanzawa K. FEBS Lett. 1976; 72:323–326. [PubMed: 16386050]
5. a) Khaarwar RN, Mishra A, Taneja SK, Stierle A, Steie D. Nat Prod Rep. 2011; 28:1208–1228. [PubMed: 21455524] b) Zhang HW, Song YC, Tan RX. Nat Prod Rep. 2006; 23:753–771. [PubMed: 17003908]
6. Huang P, Li CS, Sarotti AM, Turkson J, Cao S. Tetrahedron Lett. 2017; 58:1330–1333.
7. Li CS, Ding Y, Yang BJ, Gabriella M, Yin HQ, Walker L, Fenstemacher R, Turkson J, Cao S. Org Lett. 2015; 17:3556–3559. [PubMed: 26107089]
8. Li CS, Yang BJ, Fenstemacher R, Turkson J, Cao S. Tetrahedron Lett. 2015; 56:1724–1727.
9. Li CS, Ding Y, Yang BJ, Hoffman N, Yin HQ, Mahmud T, Turkson J, Cao S. Phytochemistry. 2016; 126:41–46. [PubMed: 26995148]
10. Li CS, Ren G, Yang BJ, Miklossy G, Turkson J, Fei P, Ding Y, Walker LA, Cao S. Org Lett. 2016; 18:2335–2338. [PubMed: 27135759]
11. 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 i550FT-IR spectrometer, respectively. NMR spectra including 1D and 2D experiments were recorded in methanol-d4 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 Gardon 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), KH2PO4 (0.5 g/L), MgSO4·7H2O (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 filter paper to separate the supernatant from the mycelia. The supernatant solution was passed through Diaion HP-20 eluted with MeOH–H2O (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 × 21.2 mm; 10 mL/min; with 0.1% formic acid in mobile phases) eluted with 20–50% MeOH–H2O in 30 min to get 30 sub-fractions (B1–B30). B18 (7.20 mg) was purified by semi-preparative HPLC (C18 column, 250.0 × 10.0 mm; 3 mL/min; with 0.1% formic acid in 13% CH3CN/H2O) to obtain compound 1 (2.75 mg, tR 33.4 min). (1) white powder; [α] +9.4 (c 0.32, MeOH); UV (MeOH) λmax 203, 268 nm; IR (film) νmax 3335, 2955, 2870, 2360, 2342, 1723, 1653, 1605, 1559, 1457, 1437, 1397, 1375, 1358, 1253 cm−1; 1H NMR (400 MHz, CD3OD) and 13C NMR (100 MHz, CD3OD): see Table 1; HRESIMS m/z 261.1336 [M+H2O−H]+ (calcd for C12H21O6, 261.1338). e) Reaction with Mosher reagents: Acylation of compound 1 (0.7 mg, 2.87 μM each) with S- (+) and R-(−)-α-methoxy-α-(trifluoromethyl) phenyl acetyl chloride (MTPA-Cl) (13) (21.43 μM each) yielded 2′-MTPA esters 1a (1.26 mg, 1.87 μM) and 1b (1.32 mg, 1.97 μM).
1.95 μM), respectively (Fig. 3). The $^1$H NMR signals of the MTPA esters were assigned on the basis of their COSY spectra, and the $\delta_{\text{T}(S-R)}$ values were then calculated (Fig. 3).  

12. Bister B, Bischoff D, Nicholson GJ, Valdebenito M, Schneider K, Winkelmann G, Hantke K, Süßmuth RD. Biometals. 2004; 17:471–481. [PubMed: 15259369]

13. a) Cao S, Guza RC, Wisse JH, Miller JS, Evans R, Kingston DGI. J Nat Prod. 2005; 68:487–492. [PubMed: 15844934] b) Hoye TR, Jeffrey CS, Shao F. Nat Proctoc. 2007; 2:2451–2458.

14. a) Grimblat N, Sarotti AM. Chem Eur J. 2016; 22:12246. [PubMed: 27405775] b) Lodewyk MW, Siebert MR, Tantillo DJ. Chem Rev. 2012; 112:1839. [PubMed: 22091891] c) Zanardi MM, Suárez AG, Sarotti AM. J Org Chem. 2017; 82:1873. [PubMed: 28209666] d) Zanardi MM, Sarotti AM. J Org Chem. 2015; 80:9371. [PubMed: 26339863]

15. a) Grimblat N, Kaufman TS, Sarotti AM. Org Lett. 2016; 18:6420. [PubMed: 27978653] b) Novaes LFT, Sarotti AM, Pili RA. J Org Chem. 2015; 80:12027. [PubMed: 26513545] c) Sarotti AM, Suárez AG, Spanevello RA. Tetrahedron Lett. 2011; 52:3116.

16. Grimblat N, Zanardi MM, Sarotti AM. J Org Chem. 2015; 80:12526. [PubMed: 26580165]

17. Ansari AA, Rajasekaran P, Khan MM, Vankar YD. J Org Chem. 2014; 79:1690–1699. [PubMed: 24456236]

18. Ogawa S, Ohno M, Ohhira T. Heterocycles. 1999; 50:57–62.

19. Georgian, V. US. 2853814 19581028. 1958.
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

Key: (a) (R)-MTPA-Cl or (S)-MTPA-Cl, pyridine, rt, 12 h.

$$\Delta \delta = \delta^{(1b)} - \delta^{(1a)}$$
Figure 4.
Proposed fragmentation mechanism of compound 1
Figure 5.
Proposed biosynthetic pathway for compound 1
Figure 6.
Some multiple hydroxylated octahydro-2H-chromenes
Table 1

NMR Spectroscopic Data for $\mathbf{1}$ in MeOH-$d_4$

| no. | $\delta^a$ (Hz) $\mathbf{1}$ | $\delta^b$ (Hz) $\mathbf{1}$ | ROESY correlations |
|-----|------------------------------|------------------------------|--------------------|
| 1   | 212.4                        |                              |                    |
| 2   | 4.24, dd, 12.0, 6.7          | 76.5                         | 6                  |
| 3   | 1.90, 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, dddd, 11.7, 9.3, 2.6   | 81.9                         | 5, 7, 10a, 10b     |
| 10  | 1.38, m                      | 35.3                         |                    |
| 11  | 1.35, m                      | 19.7                         | 11a-3              |
| 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.
## Table 2

Calculated $^1$H and $^{13}$C NMR shifts of 1

| no. | Exp $\delta_{H}$ | Calc $\delta_{H}$ | Exp $\delta_{C}$ | Calc $\delta_{C}$ |
|-----|------------------|------------------|------------------|------------------|
| 1   | 4.24             | 4.32             | 76.5             | 73.7             |
| 2   | 1.90             | 2.03             | 3.14             | 2.25             |
| 3   | 2.03             | 2.02             | 2.92             | 2.86             |
| 4   | 3.97             | 4.02             | 79.4             | 77.2             |
| 5   | 3.15             | 3.00             | 55.2             | 51.7             |
| 6   | 3.49             | 3.38             | 75.6             | 73.8             |
| 7   | 3.63             | 3.43             | 72.7             | 70.4             |
| 8   | 3.08             | 3.21             | 81.9             | 77.6             |
| 9   | 1.38             | 1.32             | 1.78             | 1.79             |
| 10  | 1.35             | 1.36             | 1.49             | 1.58             |
| 11  | 0.92             | 0.90             | 14.6             | 14.1             |

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