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Vulgarisin A, a New Diterpenoid with a Rare 5/6/4/5 Ring Skeleton from the Chinese Medicinal Plant *Prunella vulgaris*

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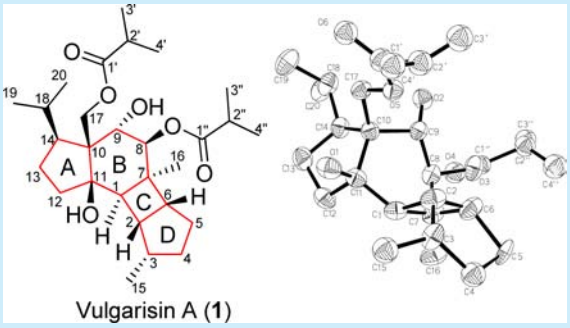
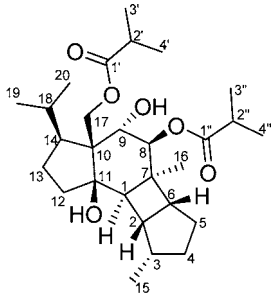
Supporting Information

ABSTRACT: Vulgarisin A (**1**), a new diterpenoid with an unprecedented 5/6/4/5 fused tetracyclic ring skeleton, has been isolated from the medicinal plant *Prunella vulgaris* Linn. Its structure was characterized by extensive spectroscopic methods, and the absolute configuration was secured by single crystal X-ray diffraction analysis. Compound **1** showed weak cytotoxicity against human lung carcinoma A549 cells with an IC_{50} value of 57.0 μM.



Prunella vulgaris Linn. (Labiatae) is a traditional Chinese medicinal herb mainly distributed in temperate Eurasia and mountainous regions of the tropics. This plant has been used as a dietary supplement and in the main contents of many famous commercial soft drinks in China.[1] Previous phytochemical investigations of *Prunella* have resulted in the isolation of triterpenes,[2] coumarins,[2] sterols,[3] flavonoids,[4] and organic acids,[5] which were associated with antibacterium,[6] anti-inflammatory,[7] anti-HIV,[8] anticancer,[9] and other biological activities.[10] Although diterpenes represent a large and structurally diverse family of bioactive natural products,[11,12] there is only one acyclic diterpene (*trans*-phytol) that has been isolated from the genus of *Prunella*.[13] During an ongoing search for bioactive constituents from *P. vulgaris* Linn. grown in the Guizhou Province of China, a new diterpenoid named vulgarisin A (**1**) has been isolated from the crude ethanolic extracts of the whole plant. Spectroscopic and X-ray crystallographic methods suggested that vulgarisin A (**1**) represented a new diterpenoidal carbon skeleton possessing a rare 5/6/4/5 fused tetracyclic ring architecture. This paper describes the isolation, structural elucidation, and plausible biosynthetic pathway of **1** as well as its cytotoxicities against two human carcinoma cell lines.

Vulgarisin A (**1**) was obtained as colorless lumpish crystals (MeOH).[14] Its molecular formula, $C_{28}H_{46}O_6$, was deduced by positive HR-ESI-MS at m/z 501.3183 $[M + Na]^+$ (calcd 501.3192 for $C_{28}H_{46}O_6Na$), indicating six degrees of unsaturation. The IR absorption bands at 3483 and 1713 cm^{-1} indicated the presence of hydroxy and carbonyl functionalities, respectively. In the ^1H NMR spectrum (Table 1), the signals of three isopropyl groups at (δ_H 0.91, 3H, d, J = 6.8 Hz; δ_H 1.01, 3H, d, J = 6.8 Hz; δ_H 2.12, 1H, m); (δ_H 1.22, 3H, d, J = 7.2 Hz; δ_H 1.19, 3H, d, J = 7.2 Hz; δ_H 2.62, 1H, m); and (δ_H 1.20, 3H, d, J = 7.2 Hz; δ_H 1.18, 3H, d, J = 7.2 Hz; δ_H 2.59, 1H, m) were clearly apparent. The ^{13}C NMR and DEPT spectra of **1** (Table 1) revealed 28 carbon signals, including eight methyls, five methylenes, ten methines, and five quaternary carbons. Among them, an oxyquaternary carbon (δ_C 82.8), two oxymethine (δ_C 76.9, δ_C 70.2), and an oxymethylene (δ_C 64.4) were observed. In addition, the presence of two carbonyl groups could be easily identified from the ^{13}C NMR signals at δ_C 179.1 and 176.8, respectively (Table 1).



In addition, the HMBC correlations between the two isopropyl substitutions (δ_H 1.22, 1.19, 2.62 and δ_H 1.20, 1.18, 2.59) and the two carbonyl carbons, respectively, indicated the existence of two isobutyryl groups. These observations, together with the molecular formula, revealed that vulgarisin A (**1**) should be a diterpenoid possessing a tetracyclic aliphatic ring skeleton.

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Table 1. Experimental NMR Chemical Shifts of 1 (δ in ppm, J in Hz)[a]

position	δ_H, mult, intrgt (J)	δ_C (mult)
1	1.88, d, 1H (5.2)	47.7 (CH)
2	2.75, m, 1H	41.2 (CH)
3	1.85, m, 1H	36.8 (CH)
4α	1.74, m, 1H	34.1 (CH_2)
4β	1.29, m, 1H	
5α	1.68, m, 1H	42.4 (CH_2)
5β	1.46, m, 1H	
6	2.74, m, 1H	38.1 (CH)
7		38.2 (qC)
8	5.09, d, 1H (11.2)	76.9 (CH)
9	4.46, dd, 1H (7.6, 11.2)	70.2 (CH)
10		56.0 (qC)
11		82.8 (qC)
12	1.60, m, 2H	26.8 (CH_2)
13	1.62, m, 2H	23.5 (CH_2)
14	2.22, m, 1H	51.8 (CH)
15	0.93, d, 3H (8.4)	14.1 (CH_3)
16	0.95, s, 3H	20.9 (CH_3)
17α	4.65, d, 1H (10.8)	64.4 (CH_2)
17β	4.28, d, 1H (10.8)	
18	2.12, m, 1H	27.2 (CH)
19	1.01, d, 3H (6.8)	25.6 (CH_3)
20	0.91, d, 3H (6.8)	20.4 (CH_3)
1′		179.1 (qC)
2′	2.62, m, 1H	34.3 (CH)
3′	1.22, d, 3H (7.2)	19.2 (CH_3)
4′	1.19, d, 3H (7.2)	18.9 (CH_3)
1″		176.8 (qC)
2″	2.59, m, 1H	34.3 (CH)
3″	1.20, d, 3H (7.2)	19.1 (CH_3)
4″	1.18, d, 3H (7.2)	18.8 (CH_3)
9-OH	2.01, d, 1H (7.6)	
11-OH	1.81, s, 1H	

[a]Data recorded in $CDCl_3$ at 400 MHz for ^1H and 100 MHz for ^{13}C NMR.

Interpretation of the 2D NMR data, including $^1H-^1H$ COSY, HMQC, HMBC, and NOESY spectra, led to the construction of the planar structure of **1**. The COSY spectrum of **1** suggested the presence of three individual spin systems (C-1, 2, 3, 4, 5, 6 to C-15), (C-8 to C-9, including 9-OH), and (C-13, 14, 18, 19, 20 to C-12) (Figure 1). The HMBC correlations from H-17α (δ_H 4.65) and H-2′ (δ_H 2.62) to C-1′ (δ_C 179.1); and from H-8 (δ_H 5.09) and H-2″ (δ_H 2.59) to C-1″ (δ_C 176.8) indicated the connection of C-2′ to C-17 and C-2″ to C-8 through the carbonyl carbon, respectively. Key HMBC correlations from H-1 (δ_H 1.88), H-8 (δ_H 5.09) to C-7 (δ_C 38.2); from H-9 (δ_H 4.46), H-14 (δ_H 2.22), H-17 (δ_H 4.65, 4.28), H-18 (δ_H 2.12) to C-10 (δ_C 56.0); and from H-1 (δ_H 1.88), H-2 (δ_H 2.75), H-12 (δ_H 1.60), H-13 (δ_H 1.62) to C-11 (δ_C 82.8) indicated that the three spin systems were connected by C-7, C-10, and C-11, respectively. In addition, the HMBC correlations from Me-16 (δ_H 0.95) to C-1 (δ_C 47.7), C-6 (δ_C 38.1), and C-8 (δ_C 76.9) were detected. These data led to the planar structure of vulgarisin A (**1**) as shown in Figure 1.

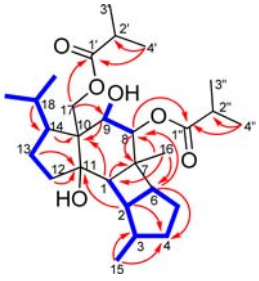

Figure 1. Key $^1H-^1H$ COSY (bold) and HMBC (arrow) correlations of **1**.

A NOESY experiment was also performed to elucidate the relative configuration of **1** (Figure 2). In the NOESY spectrum, the NOE correlations of H-2/H-9, H-6/H-9, H-6/11-OH, 11-OH/H-17β indicated that they were cofacially oriented, whereas the NOE correlations of 9-OH/H-1, 9-OH/H-8, H-8/H-14, H-8/Me-15, and H-8/Me-16 showed that they were oriented in the other direction.

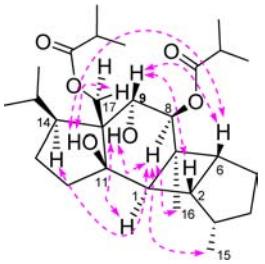

Figure 2. Selected NOESY correlations (double arrows) of **1**.

To confirm the planar structure and its absolute configuration, a single-crystal X-ray diffraction experiment of vulgarisin A (**1**) was performed by using Cu Kα radiation (Figure 3).[15] The absolute configuration of **1** was determined as 1*R*, 2*S*, 3*S*, 6*R*, 7*S*, 8*S*, 9*S*, 10*S*, 11*R*, and 14*S* through the refinement of Flack's parameter [χ = 0.0(3)].[16,17] Thus, the absolute structure of **1** with its new carbon skeleton was unambiguously established.

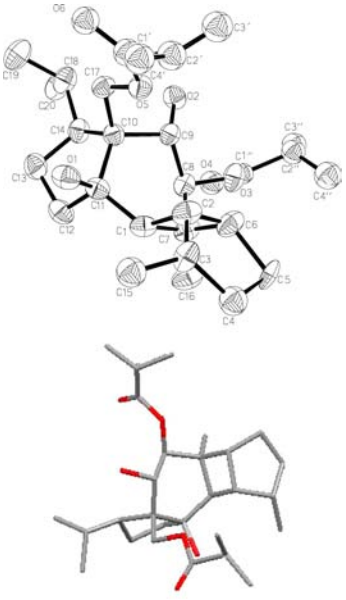

Figure 3. X-ray crystallographic structure of **1**.

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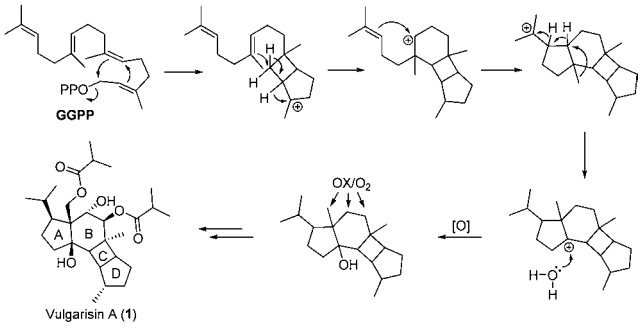
Vulgarisin A (**1**) is a new diterpenoid containing a 5/6/4/5 fused tetracyclic ring system with a core tricyclo [6−4−5] fused ring framework. To the best of our knowledge, this is the first description of this carbon skeleton found in the natural kingdom. We propose that the named "vulgarisane" be used for this new diterpenoidal skeleton type. Though quite a few diterpenes with a bicyclo [4−5] ring motif have been identified in natural compounds,[18−21] natural diterpenes containing this core tricyclo [6−4−5] ring architecture have never been reported so far. Moreover, compound **1** represented the first cyclic diterpenoid isolated from the plant genus *Prunella*.

A plausible biogenetic pathway for vulgarisin A (**1**) is shown in Scheme 1. It is rationally derivable from geranylgeranyl diphosphate (GGPP) and may subsequently involve cyclization, migration, oxidation, and esterification biosynthetic processes.[22] We postulate that initial head-to-tail cyclization of GGPP produces a bicyclo [4−5] fused ring framework (ring C and D).[19] This intermediate may later involve H-migration and cyclization of the 6- (ring B) as well as the 5-member (ring A) ring moieties, respectively, to generate the elaborate 5/6/4/5 fused tetracyclic diterpene precursor. Oxidization and esterification of this precursor is envisioned to afford the title compound **1**.

Scheme 1. Plausible Biogenetic Pathway of 1



Vulgarisin A (**1**) was investigated for its cytotoxicities against two human carcinoma cell lines.[23] The results showed that **1** possessed a weak cytotoxicity against human lung carcinoma A549 cells with an IC_{50} value of 57.0 μM (the positive control 5-fluorouracil showed an IC_{50} value of 7.6 μM) but no obvious cytotoxicity against human cervical carcinoma HeLa 60 cells. Interestingly, removal of the two hydrophobic isobutyryl substituents increased the inhibitory activity 3-fold (IC_{50} = 20.5 μM) against A549 cells.

■ ASSOCIATED CONTENT

Supporting Information

1D, 2D NMR, MS, UV, IR, and CD spectra, detailed isolation experimental procedures and X-ray diffraction data (CIF file) of **1**, the synthetic procedure for the deprotected derivative of **1**, as well as the cytotoxicities of both molecules against two human carcinoma cell lines. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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(14) Vulgarisin A (**1**): Colorless lumpish crystals, mp 211−213 °C (MeOH); $[\alpha]^{20}_{D}$ +2.59 (*c* 1.07, $CHCl_3$); CD (CH_3CN) (215 nm, $\Delta\varepsilon$ +0.48); UV ($CHCl_3$) λ_{max} 241 nm; IR (KBr) ν_{max} 3483, 2957, 1713, 1467, 1393, 1217, 1165, 1084 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; positive ESI-MS *m/z* 479 $[M + H]^+$, 501 $[M + Na]^+$; positive HR-ESI-MS *m/z* 501.3183 $[M + Na]^+$ (calcd 501.3192 for $C_{28}H_{46}O_6Na$).
(15) Crystals of vulgarisin A (MeOH) belong to the trigonal space group $P3_1$. The crystal data: $C_{28}H_{46}O_6$, M = 478.67, a = 12.898 (4) Å, b = 12.898 (4) Å, c = 15.364 (8) Å, $\alpha = \beta = 90°$, $\gamma = 120°$, V = 2213.5(15) $Å^3$, Z = 3, d = 1.018 g/cm^3. A crystal of dimensions 0.30 × 0.36 × 0.53 mm^3 was used for measurements on a Rigaku MicroMax 002+ diffractometer with focusing monochromator ($\omega-\kappa$ scans, 2θ max = 135.98°), Cu Kα radiation. The total number of independent reflections measured was 4013, of which 3295 were observed ($|F|^2 \geq 2\sigma|F|^2$). The crystal structure was solved and refined by the direct method Shelxs-97, expanded using difference Fourier techniques and full-matrix least-squares calculations. Final indices: R_1 = 0.0687, wR_2 = 0.1942, S = 1.088. Crystallographic data for the structure of **1** have been deposited in the Cambridge Crystallographic Data Centre (Deposition No. CCDC986630). These data can be obtained free of charge via www.ccdc.com.ac.uk/conts/retrieving.html (or 12 Union

Road, Cambridge CB21EZ, U.K.; Fax: (+44)1223-336-033; E-mail: deposit@ccdc.cam.ac.uk).
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