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A New Monoterpene Alkaloid From the Stems of *Rauvolfia vomitoria*

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Abstract: One new monoterpene alkaloid (**1**) and eight known compounds (**2**–**9**), belonging to two monoterpene alkaloids (**1**–**2**), one pyridine alkaloid (**3**), four phenylpropanoid derivatives (**4**–**7**), and two matrine-type alkaloids (**8**–**9**), were isolated from *Rauvolfia vomitoria* Afzel. The structure of new compound **1** was determined by extensive spectroscopic analysis and electronic circular dichroism (ECD) calculation. Notably, this is the first reported monoterpene alkaloids from *R. vomitoria*. Besides, compounds **3**, **5**, and **7**–**9** were first discovered in the Apocynaceae family and compounds **4** and **6** were first reported in the *Rauvolfia* genus. This is also the first example of matrine-type alkaloids reported in the Apocynaceae family, indicating that matrine-type alkaloids are not unique to the Leguminosae/Fabaceae family. All isolated compounds were evaluated for their anti-acetylcholinesterase, anti-α-glucosidase, and antioxidant activities. Compound **6** showed significant α-glucosidase inhibitory activity and remarkable DPPH radical scavenging capacity, both of which are superior to the positive controls. Molecular docking of compound **6** with α-glucosidase was further performed, suggesting that compound **6** could form hydrogen bonds with residues ALA-292, ASN-259, and ARG-263.

Keywords: *Rauvolfia vomitoria*; AChE; DPPH; α-glucosidase; monoterpene alkaloids; molecular docking.

1. Introduction

Rauvolfia vomitoria Afzel., belonging to the family Apocynaceae, is native to tropical Africa and widely cultured in the south of China, such as Yunnan, Guangxi, and Guangdong province [1]. This plant is a famous "southern medicine" in China, used to treat hypertension, high fever, epilepsy, and pain [1].

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Previous phytochemical studies on this plant mainly focused on the monoterpene indole alkaloids (MIAs), which exhibited potential anti-inflammatory, cytotoxicity, and acetylcholinesterase inhibitory activities [1–3]. However, few studies paid attention to other types of alkaloids, especially monoterpene alkaloids, in this plant.

The course of a search for bioactive natural products with structural diversity in *R. vomitoria* led to the isolation of nine compounds including a new monoterpene alkaloid (**1**) and eight known compounds (**2**–**9**). The structure of the new compound (**1**) was determined by 1D and 2D NMR, HRESIMS, and electronic circular dichroism (ECD), as well as ECD calculation. Interestingly, it was first reported monoterpene alkaloids in *R. vomitoria*. Meanwhile, compounds **4** and **6** were reported from this genus for the first time. Herein, the isolation, structure elucidation, anti-AChE activity, anti-α-glucosidase, and antioxidant activities were explored.

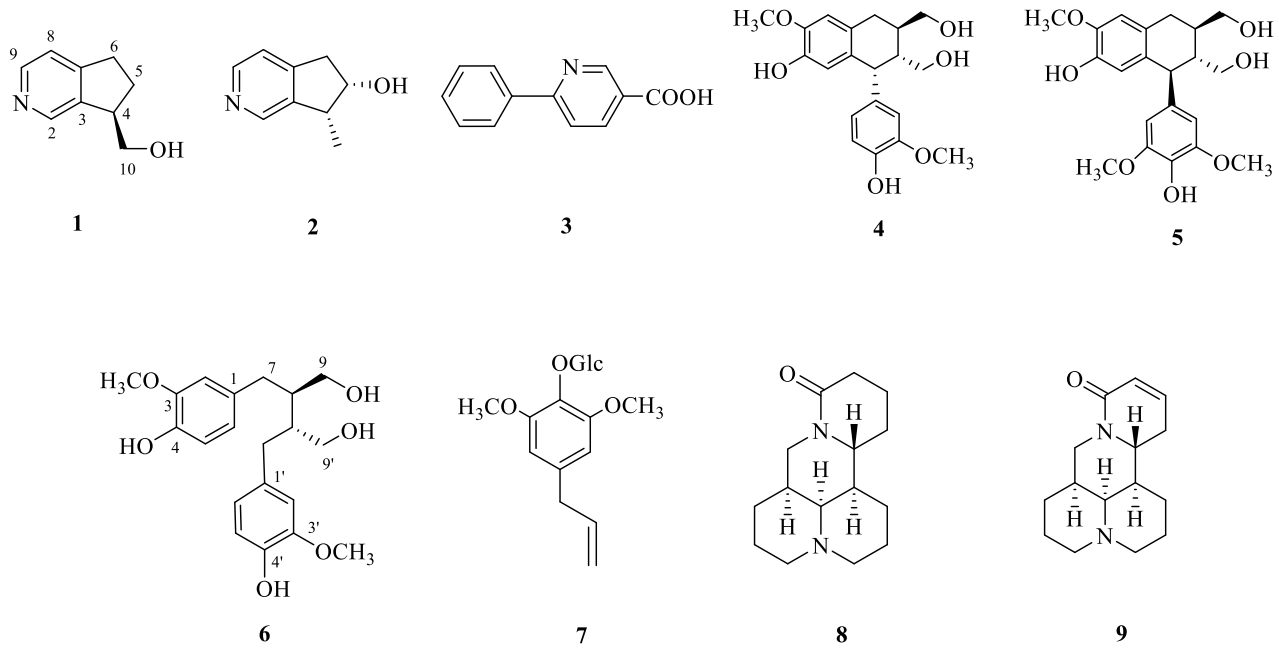

Figure 1. Chemical structures of compounds **1**–**9**

2. Materials and Methods

2.1. General Experimental Procedures

Column chromatography (CC) was carried out using different fillers, including silica gel (Qingdao Haiyang Chemical Co. Ltd., China), ODS (45 μm, YMC Co. Ltd., Japan), and Sephadex LH-20 (GE Healthcare Bio-Sciences AB, Sweden). TLC was performed by silica gel 60 F254 (Yantai Chemical Industry Research Institute). HPLC was conducted on Waters 1525 apparatus with YMC Triart C18 column (5 μm, 10 × 250 mm). 1D and 2D NMR spectra were recorded by a Bruker Avance III HD-600. Optical rotations were examined in methanol by an InsMark FD polarimeter. HRESIMS spectra were obtained from a Waters I-Class Vion IMS QTOF spectrometer. UV and FT-IR spectra were recorded using Agilent Cary 60 instrument and Thermo Fisher Niclolet 6700 instrument, respectively.

2.2. Plant Material

The stems of *R. vomitoria* were collected in August 2017 from Xishuangbanna, Yunnan Province, P. R. China, and identified by Dr. Tao Zhou at this institute. A voucher specimen (No. 20170817) has been deposited at this institute.

2.3. Extraction and Isolation

The dried and crushed stems of *R. vomitoria* (50 Kg) were extracted with 80% methanol at 40°C (120 L × 4, each for 2 days). The crude extract was suspended in water, then acidified to pH 2 with a dilute HCl solution. After extracted with $CHCl_3$, the acid water layer was alkalified to pH 9 with Na_2CO_3 solution. Then extraction with $CHCl_3$ to obtain the $CHCl_3$ extract (63.7 g), which was subjected to ODS CC eluting with a gradient of CH_3OH/H_2O (from 20:80 to 90:10) to generate four main subfractions *Fr.1–Fr.4*. *Fr.1* (2.43 g) was purified by Sephadex LH-20 CC (CH_3OH) and semi-preparative reversed-phase (RP) HPLC ($CH_3OH/H_2O/Et_2NH$, 32:68:0.2) to provide **2** (14.9 mg, t_R = 29.2 min). *Fr.2* (570 mg) was purified by Sephadex LH-20 CC (CH_3OH), silica gel CC (CH_2Cl_2/ CH_3OH, 30:1), and RP HPLC ($CH_3CN/H_2O/Et_2NH$, 16:84:0.2) to yield **1** (2.4 mg, t_R = 26.7 min). *Fr.3* (1.6 g) was subjected to silica gel CC using a gradient system of CH_2Cl_2/CH_3OH (from 30: 1 to 10:1) to obtain four subfractions (*Fr.3.1–Fr.3.4*). *Fr.3.1* (150 mg) was fractionated via Sephadex LH-20 CC (CH_3OH), silica gel CC (CH_2Cl_2/CH_3OH, 30:1), and RP HPLC ($CH_3OH/H_2O/Et_2NH$, 60:40:0.2) to obtain **3** (2.0 mg, t_R = 13.5 min). **4** (1.9 mg, t_R = 43.3 min) and **5** (1.3 mg, t_R = 48.0 min) were isolated from *Fr.3.2* (476 mg) by Sephadex LH-20 CC (CH_3OH) and RP HPLC ($CH_3OH/H_2O/Et_2NH$, 25:75:0.2). **6** (3.1 mg, t_R = 27.6 min) was also obtained from *Fr.3.2* by Sephadex LH-20 CC (CH_3OH) and RP HPLC ($CH_3CN/H_2O/Et_2NH$, 20:80:0.2). *Fr.3.3* (295.7 mg) was fractionated on Sephadex LH-20 CC (CH_3OH) to afford two subfractions, *Fr.3.3.1 and Fr.3.3.2.* Purification of *Fr.3.3.1* by RP HPLC ($CH_3OH/H_2O/Et_2NH$, 50:50:0.2) afford compound **7** (10 mg, t_R =26.6 min). *Fr.3.3.2* was purified using RP HPLC ($CH_3OH/H_2O/Et_2NH$, 60:40:0.2) to yield compounds **8** (4.3 mg, t_R = 22.1 min) and **9** (2.2 mg, t_R = 23.9 min). The flow rates of all the HPLC experiments were 2.0 mL/min.

*Compound **1***: Colorless oil. $[\alpha]_D^{20} = -70$ (c 1.00, $CHCl_3$). FT-IR ν_{max} 3213, 2922, 2858, 1599, 1570, 1481, 1456, 1421, 1369, 1178, 1076, 1042, 1007, 829, 729 cm^{-1}. UV (CH_3OH) λ_{max} (log ε): 258 (3.2) nm. ECD (CH_3OH) 272 (Δε, +0.79), 251 (Δε, +0.79), 231 (Δε, +1.48) nm. HRESIMS *m/z* 150.08922 $[M + H]^+$ (calculated for $C_9H_{12}NO^+$, 150.08742). ^{1}H-NMR (CD_3OD, 600 MHz) and ^{13}C-NMR (CD_3OD, 150 MHz) see Table 1.

Table 1. ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR data of **1** in CD_3OD

Position	δ_C	δ_H	Position	δ_C	δ_H
2	146.3	8.47, s	6	32.3	2.93, ddd (17.0, 8.7, 6.7)
3	143.2				3.01, ddd (17.0, 8.8, 6.1)
4	47.3	3.40, ddd (14.3, 6.5, 6.5)	7	157.0	
5	29.1	2.28, dddd (12.7, 8.7, 6.5, 6.1)	8	121.9	7.31, br d (5.0)
		1.93, dddd (12.7, 8.8, 6.7, 6.5)	9	148.0	8.28, d (5.0)
			10	65.9	3.70, 2H, overlapped

2.4. Acetylcholinesterase Inhibition Assay

Acetylcholinesterase inhibition assay was performed as previously described [4]. Galanthamine was used as a positive control in this assay.

2.5. α-Glucosidase Inhibitory Assay

The α-glucosidase assay was performed with slight modification [5]. Acarbose was used as a positive control.

2.6. Antioxidant Capacity Assay

The antioxidant capacity of isolated compounds was evaluated by 1,1 -diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging assay [6], Ferric reducing antioxidant power (FRAP) assay [7], and hydroxyl radical scavenging assay [7]. Ascorbic acid was used as a positive control.

2.7. Molecular Docking Studies

The crystallographic structure of α-glucosidase (PDB code: 3A4A) was downloaded from www.rcsb.org/ The structure of compound **6** in *Mol2* format was prepared. Molecular docking was completed using *Autodock 4.0*. It was set as a receptor after being removed water molecules in this protein, then made a hydrogenation operation. After hydrogenation and charge calculation with compound **6**, the optimal structure conformation was chosen as a ligand. The protein remaining stationary was placed in a stereoscopic box with *X*, *Y*, and *Z* axis dimensions of 126 Å, while the ligands could move flexibly. The algorithm GA run for ten times and other values were set as default. The docking model with the lowest binding energy was selected as the optimal result.

3. Results and Discussion

3.1. Structure Elucidation

Compound **1** was isolated as a colorless oil. Its HRESIMS iron peak $[M + H]^+$ at *m/z* 150.08922 established the molecular formula of $C_9H_{11}NO$ (calculated for $C_9H_{12}NO^+$, 150.08742) indicating five degrees of unsaturation. The absorption band at 3213 cm^{-1} in the IR spectrum suggested the presence of a hydroxyl group. The absorption peak at 258 nm in the UV spectrum may be the characteristic absorption of the pyridine ring [8]. The ^1H NMR data (Table 1) provided three aromatic protons at δ_H 8.47 (1H, s, H-2), 8.28 (1H, d, J = 5.0 Hz, H-9), 7.31 (1H, br d, J = 5.0 Hz, H-8), a pair of oxymethylene protons at δ_H 3.70 (2H, overlapped, H-10), two pairs of methylene protons at δ_H 2.28 (1H, dddd, J = 12.7, 8.7, 6.5, 6.1 Hz, H-5a), 1.93 (1H, dddd, J = 12.7, 8.8, 6.7, 6.5Hz, H-5b), 2.93 (1H, ddd, J = 17.0, 8.7, 6.7 Hz, H-6a), 3.01 (1H, ddd, J = 17.0, 8.8, 6.1Hz, H-6b), and one methine proton at δ_H 3.40 (1H, ddd, J = 14.3, 6.5, 6.5 Hz, H-4). The ^{13}C-NMR data of **1** (Table 1) suggested the presence of five aromatic carbon signals at δ_C 146.3 (C-2), 143.2 (C-3), 157.0 (C-7), 121.9 (C-8), 148.0 (C-9), three methylene groups including one oxymethylene group at δ_C 65.9 (C-10), and one methine group at δ_C 47.3 (C-4). The above information indicated the presence of a pyridine ring in **1**. The pyridine ring accounts for four degrees of unsaturation,

and the remaining one degree of unsaturation suggested the presence of an additional ring system in **1**. Therefore, compound **1** belongs to monoterpene alkaloids. The connection of "CH_2(10)-CH(4)-CH_2(5)-CH_2(6)" could be determined by the ^{1}H-^{1}H COSY correlations as shown in Figure 2. The HMBC correlations (Figure 2) from H-6 to C-7, C-8, and C-3, and from H-4 to C-2, C-3, and C-7 indicated that C-6 was linked to C-7, and C-4 was connected to C-3. Thus, the plane structure of **1** was determined as shown in Figure 2.

To determine the absolute configuration of **1**, the ECD spectrum was calculated based on the method of time-dependent density functional theory (TD-DFT) as described previously [4]. As shown in figure 3, the experimental ECD spectrum of **1** fits well with the calculated ECD spectrum of 4*R*-**1**. Thus, the structure of **1** was ultimately identified as 4*R*-**1**, named (*R*)-10-hydroxyl-4-noractinidine. This is the first time report of monoterpene alkaloids from *R. vomitoria.*

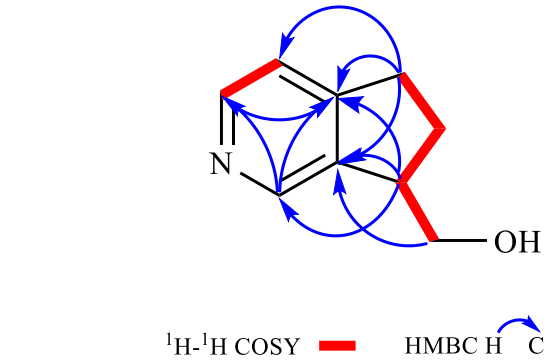

Figure 2. The key ^{1}H-^{1}H COSY and HMBC correlations for **1**

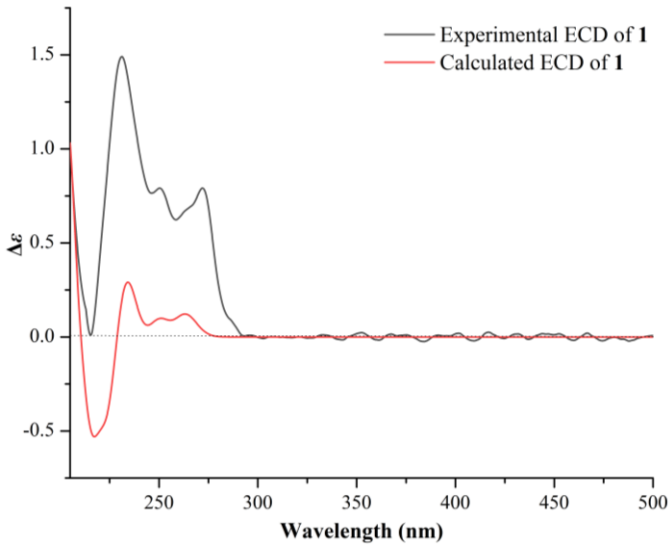

Figure 3. The experimental and calculated ECD of **1**

The known compounds (**2**–**9**) were identified as venoterpine (**2**) [9], 6-phenylnicotinic acid (**3**) [10], (+)-isolariciresinol(**4**) [11], (+)-5-methoxyisolariciresinol (**5**) [12], (–)-secoisolariciresinol (**6**) [13], 4-allyl-2,6-dimethoxyphenol glucoside (**7**) [14], matrine (**8**) [15], and sophocarpine (**9**) [16] by comparison with the literature data. Notably, compounds **3**, **5**, and **7**–**9** were first discovered in the Apocynaceae family. Compounds **4** and **6** were first reported from the *Rauvolfia* genus, enriching the structural diversity of this genus. Quinolizidine alkaloids were thought to be the characteristic compounds in Leguminosae/Fabaceae family [17]. Compounds **8** and **9** were the first examples of quinolizidine alkaloids reported in the Apocynaceae family, indicating that quinolizidine alkaloids are not unique to the Leguminosae/Fabaceae

family.

3.2. Antioxidant, Acetylcholinesterase, α-glucosidase Inhibitory Activity

The *n*-hexane and methanol extracts of the leaves of *R. tetraphylla*, another species of this genus, showed significant antioxidant activity [18]. Thus, the antioxidant activities of isolated compounds were evaluated. As shown in Table 2, only compounds **4**–**6** showed significant DPPH scavenging capacity with IC_{50} values of 39.40, 65.97, and 15.06 μM, respectively, while other compounds did not show potent DPPH scavenging capacity (IC_{50} > 200 μM). Interestingly, the DPPH scavenging capacity of compound **6** (15.06 μM) was much better than the positive control, ascorbic acid (28.36 μM). Additionally, compound **6** showed greatest FRAP value (222.99 ± 0.83 μM $FeSO_4$/100 μM), followed by compound **4** (183.69 ± 0.10 μM $FeSO_4$/100 μM) and compound **5** (80.86 ± 0.25 μM $FeSO_4$/100 μM), however other compounds did not present FRAP ability (<10 μM $FeSO_4$/250 μM) at preliminary screening (250 μM). Unfortunately, compounds **1**–**9** failed to exhibit hydroxyl radical scavenging activities. All the isolated compounds **1**–**9** were also evaluated the hydroxyl radical scavenging assay at a concentration of 250 μM, however, none of them showed potential activity with inhibitory rates below 10 %.

Table 2. Antioxidant activities of all iaolated compounds **1**–**9**[a]

Compounds	DPPH radical scavenging activity[b]	FRAP value[c]	Hydroxyl radical scavenging activity[d]
	IC_{50} (μM)[d]	**μM $FeSO_4$/100 μM**	**IC_{50} (μM)**
4	39.40 ± 0.52	183.69 ± 0.10	>250
5	65.97 ± 0.09	80.86 ± 0.25	>250
6	15.06 ± 0.12	222.99 ± 0.83	>250
Ascorbic acid[e]	28.36 ± 0.09	210.39 ± 0.48	156.94 ± 2.65

[a]Values are expressed as the mean ± SD (n = 3). [b]Other compounds, IC_{50} > 200 μM. [c] Other compounds, <10 μM $FeSO_4$/250 μM. [d] Other compounds, IC_{50} > 250 μM. [e]Ascorbic acid was used as a positive control.

Previous studies have shown that some compounds in *R. vomitoria* showed acetylcholinesterase inhibitory activity, thus compounds **1**–**9** were evaluated for their anti-acetylcholinesterase activity [4]. Preliminary screening was performed at a concentration of 100 μM. Galantamine was used as a positive control (IC_{50} = 3.94 ± 0.15 μM). Unfortunately, none of them showed inhibitory rates above 10 % at 100 μM. The results suggested that MIAs may be more potential in terms of acetylcholinesterase inhibitory activity in combination with the previous study of our group [19].

Glycosylated hemoglobin and glycogen were reduced contributing to the improvement of glycemic when taking *R. serpentine*, thus compounds **1**–**9** were evaluated for their α-glucosidase inhibitory activity [20]. Compound **6** showed potent inhibition against the α-glucosidase, while other compounds showed weak inhibitory rates (<10%) through a preliminary test at a concentration of 400 μM. Further study demonstrated that compound **6** exhibited significant α-glucosidase inhibitory activity with an IC_{50} value of 182.3 ± 1.98 μM as shown in Table 2, compared with the positive control (acarbose, 330.33 ± 9.72 μM, consistent with the literature report) [5].

The inhibitory activities against α-glucosidase of compounds **4**–**6**, possessing similar structures, were significantly different, which may be caused by their diverse rotatable flexibility. Compound **6** with an open-loop structure may provide more opportunities to bind with α-glucosidase.

Table 3. α-Glucosidase inhibitory activity of compound **6** and acarbose[a]

Compounds	IC_{50} (μM)[b]
6	182.3 ± 1.98
Acarbose[c]	330.33 ± 9.72

[a]Other compounds, IC_{50} > 400 μM. [b]IC_{50} values are expressed as the mean ± SD (n = 3). [c]Acarbose was used as a positive control.

3.3. Molecular Docking Results

The lowest binding energy between compound **6** and α-glucosidase (PDB ID: 3A4A) was –4.6 Kcal/mol. As shown in Figure 4, compound **6** bound with α-glucosidase mainly through five strong hydrogen bonds with the residues ALA-292 (3.0 Å with 3-*O*CH_3, 2.5 Å with 4-OH), ASN-259 (2.0 Å with both 9-OH and 9′-OH), and ARG-263 (2.1 Å with 4′-OH). In addition, compound **6** formed hydrophobic interactions with several residues, including ILE-262, ALA-292, and MET-273. Moreover, there were also two potential π-interactions between compound **6** and the residues HIS-295 and TRP-15. The residue ASN-259 was suggested to be crucial in the stabilization of α-glucosidase complexes since it can promote thermostability [21]. ILE-262 could significantly reduce the activity of charged substrates, confirming its important role in determining the specificity of carboxypeptidase T substrates [22].

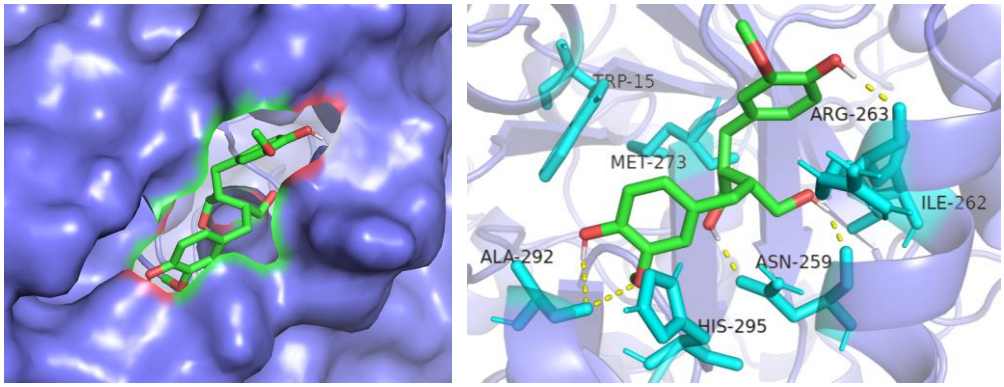

Figure 4. Docking results of compound **6**

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Supporting information

Supporting information accompanies this paper on http://www.acgpubs.org/journal/ records-of-natural-products.

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