New Indole Alkaloids from *Rauvolfia yunnanensis*

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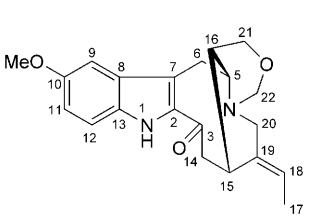
Three new indole alkaloids, 10-methoxy-16-de(methoxycarbonyl)pagicerine (**1**), (5β)-17-*O*-deacetyl-5,11-dimethoxyakuammiline (**2**), and ((16*S*,19*E*)-N^1-(hydroxymethyl)isositsirikine (**3**) were isolated from the roots of *Rauvolfia yunnanensis*, together with seven known alkaloids. The structures of the new compounds were elucidated by in-depth spectroscopic and mass-spectrometric analyses.

Introduction. – The genus *Rauvolfia* comprises *ca.* 135 species mainly distributed in America, Africa, Asia, and Oceania. Nine species and four variants grow in South China [1]. *Rauvolfia* species are rich sources of bioactive indole alkaloids such as reserpine [2]. *Rauvolfia yunnanensis* TSIANG, widely distributed in the south and southeast of China, is commonly used as a folklore herb to treat hypertension, snake bites, and insanity [3]. However, it has not been as exhaustively investigated as other *Rauvolfia* species. Due to its biological and therapeutic significance, we decided to phytochemically investigate this plant.

Herein, we report the isolation of three new indole alkaloids[1]) from *R. yunnanensis*, 10-methoxy-16-de(methoxycarbonyl)pagicerine (**1**), (5β)-17-*O*-deacetyl-5,11-dimethoxyakuammiline (**2**), and ((16*S*,19*E*)-N^1-(hydroxymethyl)isositsirikine (**3**), together with seven known alkaloids: ajmalicine [4], reserpine [5], ajmaline [6], yohimbine [4], venoterpine [7], 19-epi-ajmalicine [4], and (16*R*,19*E*)-isositsirikine [8].



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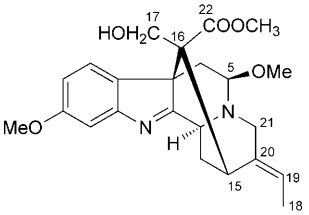

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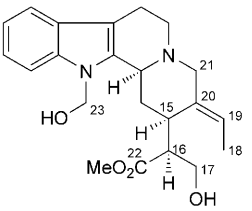

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[1]) Arbitrary atom numbering. For systematic names, see *Exper. Part.*

Results and Discussion. – Compound **1**, obtained as a yellowish amorphous solid, was optically active ($[\alpha]_D^{24} = -97$ ($c = 0.58$, C_5H_5N). Its molecular formula was determined as $C_{21}H_{24}N_2O_3$ by positive-mode HR-ESI-MS (*m/z* 353.1875 ($[M+H]^+$, calc. 353.1865)). The IR spectrum of **1** showed typical bands at 3425 (NH) and 1626 cm^{-1} (keto C=O), and the UV spectrum displayed characteristic absorptions at 209 and 322 nm, associated with a 2-acylindole moiety [9].

The ^{1}H-NMR spectrum of **1** (*Table 1*) showed three aromatic resonances at δ(H) 7.10 (*d*, $J = 2.3$ Hz), 7.06 (*dd*, $J = 2.3$, 8.8 Hz), and 7.30 (*d*, $J = 8.8$ Hz) as *ABX*-type signals. This suggested that the indole moiety was either 10- or 11-substituted[1]). The indole NH at δ(H) 9.1 (H–N(1)) showed a ROESY correlation with δ(H) 7.30 (H–C(12)), and the resonance at δ(H) 3.88 (*s*, MeO) correlated with δ(H) 7.10 (H–C(9)) and 7.06 (H–C(11)). Therefore, the MeO group was located at C(10). In the ^{1}H,^{1}H-COSY spectrum, the olefinic H-atom at δ(H) 5.36 (*q*, $J = 6.6$ Hz, H–C(18)) was coupled with a Me group at δ(H) 1.75 (*dd*, $J = 6.6$, 1.9 Hz, Me(17)) indicating an ethylidene side chain.

Table 1. *^{1}H-NMR Data for* **1**–**3**. At 400 MHz in $CDCl_3$; δ in ppm, *J* in Hz.

Position[1])	**1**	**2**	**3**
3	–	4.39 (*d*, $J = 4.8$)	4.04 (*d*, $J = 10.1$)
5	3.39–3.41 (br. *s*)	3.78–3.84 (*m*)	α: 2.85–2.87 (*m*)
			β: 2.94–2.96 (*m*)
6	α: 3.63–3.65 (*m*)	α: 2.31 (*d*, $J = 15.5$)	α: 2.77–2.79 (*m*)
	β: 3.51–3.55 (*m*)	β: 3.54 (*d*, $J = 15.5$)	β: 2.77–2.99 (*m*)
9	7.10 (*d*, $J = 2.3$)	7.43 (*d*, $J = 8.3$)	7.39 (*d*, $J = 7.6$)
10		6.69 (*dd*, $J = 8.3$, 2.4)	7.05 (*t*, $J = 7.6$)
11	7.06 (*dd*, $J = 8.8$, 2.3)		7.15 (*t*, $J = 7.6$)
12	7.30 (*d*, $J = 8.8$)	7.19 (*s*)	7.38 (*d*, $J = 7.6$)
14	α: 3.21 (*t*, $J = 12.4$)	α: 1.85 (*dd*, $J = 8.3$, 2.5)	α: 2.15–2.20 (*m*)
	β: 2.84 (*dd*, $J = 12.4$, 8.2)	β: 1.85 (*dd*, $J = 8.3$, 2.5)	β: 1.86–1.88 (*m*)
15	3.31–3.33 (*m*)	3.51–3.55 (*m*)	3.22–3.24 (*m*)
16	1.59–1.60 (br. *s*)		2.83–2.85 (*m*)
17	1.75 (*dd*, $J = 6.6$, 1.9)	2.89 (*s*)	α: 3.64–3.74 (*m*)
		2.89 (*s*)	β: 3.64–3.74 (*m*)
18	5.36 (*q*, $J = 6.6$)	1.63 (*dd*, $J = 7.0$, 1.9)	1.68 (*d*, $J = 6.5$)
19	–	5.44 (*q*, $J = 7.0$)	5.69 (*q*, $J = 6.5$)
20	α: 4.44 (*d*, $J = 16.0$)	–	–
	β: 3.45 (*d*, $J = 16.0$)		
21	α: 3.86 (*m*)	α: 3.07 (*d*, $J = 17.3$)	α: 3.29–3.33 (br. *s*)
	β: 3.77 (*dd*, $J = 11.4$, 2.4)	β: 4.06 (*d*, $J = 17.3$)	β: 3.45 (*d*, $J = 12.6$)
22	α: 4.76 (*d*, $J = 10.1$)	–	–
	β: 4.63 (*d*, $J = 10.1$)	–	–
23	–	–	5.40 (*s*)
MeO	3.87–3.89 (br. *s*, 10-MeO)	3.18 (*s*, 5-MeO)	3.62 (*s*, MeOO)
		3.82 (*s*, 11-MeO)	
		3.79 (*s*, 22-MeO)	
NH	9.1 (*s*)	–	–

The ^{13}C-NMR (DEPT) spectrum of **1** (*Table 2*) showed the presence of two Me, five CH_2, and seven CH groups, as well as seven quaternary C-atoms. The downfield shifts of a CH_2 group at δ(C) 87.9 (C(22)) suggested that it was connected both to an O- and an N-atom. In an HMBC experiment, correlations from H–C(15) to C(14) and C(5), from H–C(6) to C(2) and C(5), and from H–C(21) to C(16) and C(5) permitted the establishment of the structural fragment **a** (*Fig. 1*). Atoms C(5), C(20), and C(22) were connected each to an N-atom, based on correlations from H–C(20) to C(5) and C(22). Moreover, the correlation between H–C(22) and C(21) indicated a tetrahydro-1,3-oxazine ring, as further supported by the downfield shift of CH_2(22) [δ(H) 4.76, 4.63 (*AB* system, J = 10.1 Hz each)]. The above fragments were finally put together, based on the HMBC correlations between C(7) and H–C(6); C(3) and H–C(14), and C(19) and H–C(15), H–C(17), and H–C(20), respectively (*Fig. 1*).

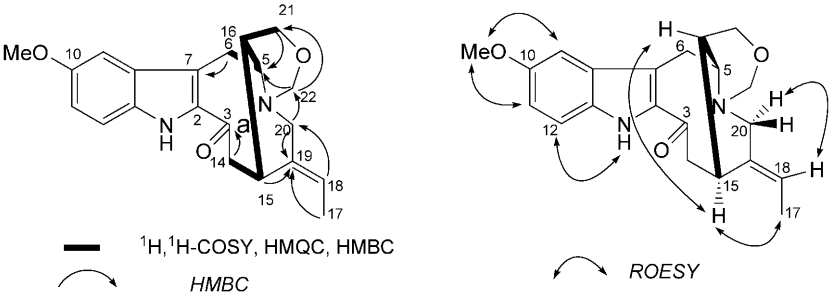

Fig. 1. *Key 2D-NMR correlations for* **1**

The configuration of **1** was determined with the aid of a ROESY experiment (*Fig. 1*). ROESY Cross-peaks were observed for the pairs H–C(15)/H–C(17) and H–C(20)/H–C(18); and the exocyclic C=C bond was assigned (*E*)-configuration [10]. On the basis of these data, and by spectroscopic comparison with literature data for pagicerine [11], amerovolficine [12], and other alkaloids of the vobasine type [9], the structure of **1** could be established. Note that the additional tetrahydro-1,3-oxazine ring in **1** is a very rare structural motive; as a matter of fact, **1** represents only the third such indole alkaloid, besides pagicerine and amerovolficine.

Compound **2**, obtained as a yellowish amorphous solid was optically active ($[\alpha]_D^{24} = -124$ (c = 0.46, C_5H_5N)). Its molecular formula was determined as $C_{23}H_{28}N_2O_5$ by positive-mode HR-ESI-MS (m/z 413.2069 ($[M+H]^+$; calc. 413.2076)). The IR spectrum of **2** showed typical bands at 3426 (OH), 2938 (C–H), 1734 (MeO_2C), and 1601 cm^{-1} (C=N–). The UV spectrum displayed characteristic absorptions at 224, 230, and 289 nm, associated with an indolenine chromophore [13].

The ^{13}C-NMR (DEPT) spectrum of **2** (*Table 2*) showed the presence of four Me, four CH_2, and seven CH groups, as well as eight quaternary C-atoms. An olefinic signal was observed at δ(C) 191.1. Based on the lowfield shift of H–C(3) at δ(H) 4.39 (*d*, J = 4.8 Hz), we expected a strictamine/akuammiline-type alkaloid with an N(1)=C(2) bond [14]. The ^{1}H-NMR spectrum (*Table 1*) showed characteristic signals of an ethylidene side chain at δ(H) 1.63 (*dd*, J = 7.0, 1.9 Hz, Me(18)) and 5.44 (*q*, J = 7.0 Hz, H–C(19)). Three aromatic resonances at δ(H) 6.69 (*dd*, J = 8.3, 2.4 Hz), 7.19 (*s*), and 7.43

Table 2. *^{13}C-NMR Data for* **1**–**3**. At 100 MHz in $CDCl_3$; δ in ppm.

Position[1])	**1**	**2**	**3**
2	135.6 (*s*)	191.1 (*s*)	133.8 (*s*)
3	191.2 (*s*)	51.3 (*d*)	51.2 (*d*)
5	56.3 (*d*)	90.7 (*d*)	47.6 (*t*)
6	26.9 (*t*)	42.8 (*t*)	20.7 (*t*)
7	118.3 (*s*)	55.2 (*s*)	109.0 (*s*)
8	128.3 (*s*)	135.4 (*s*)	126.9 (*s*)
9	100.4 (*d*)	124.8 (*d*)	118.0 (*d*)
10	154.8 (*s*)	111.5 (*d*)	119.8 (*d*)
11	118.9 (*d*)	160.3 (*s*)	121.9 (*d*)
12	113.2 (*d*)	106.7 (*d*)	109.3 (*d*)
13	131.9 (*s*)	157.4 (*s*)	137.1 (*s*)
14	44.2 (*t*)	30.7 (*t*)	30.3 (*t*)
15	34.6 (*d*)	35.3 (*d*)	34.5 (*d*)
16	32.6 (*d*)	61.6 (*s*)	51.7 (*d*)
17	11.7 (*q*)	64.7 (*t*)	61.7 (*t*)
18	116.5 (*d*)	13.5 (*q*)	13.4 (*q*)
19	137.7 (*s*)	121.0 (*d*)	126.5 (*d*)
20	50.6 (*t*)	138.2 (*s*)	132.5 (*s*)
21	74.4 (*t*)	50.7 (*t*)	58.3 (*t*)
22	87.9 (*t*)	173.1 (*s*)	174.8 (*s*)
23	–	–	66.1 (*t*)
MeO	55.7 (*q*, C(10))	54.6 (*q*, C(5)) 55.5 (*q*, C(11)) 51.9 (*q*, C(22))	51.7 (*q*, C(22))

(*d*, $J = 8.3$ Hz) were assigned to a 10- or 11-substituted indole nucleus (*ABX* spin system). The MeO resonance at δ(H) 3.82 showed a cross-peak to δ(C) 160.3 in the HMBC spectrum; and δ(H) 7.43 (H–C(9)) and 2.31 (H_α–C(6)) showed a ROESY correlation. Thus, the above first MeO group was located at C(11). The second MeO signal at δ(H) 3.79 showed an HMBC correlation with δ(C) 173.1 (C(22)), which was part of an ester group. Finally, the third MeO signal at δ(H) 3.18 showed an HMBC cross-peak with δ(C) 90.7 (C(5)), and H–C(5) at δ(H) 3.81 showed a ROESY correlation with H_α–C(21)) and H_α–C(6)) at δ(H) 3.07 and 2.31, respectively. Therefore, the third MeO group was located at C(5), and H–C(5) was α-oriented

The configuration of **2** was derived by a ROESY experiment (*Fig. 2*). Based on the cross-peaks between H_α–C(21) and H–C(19), H–C(15), and H–C(18), the ethylidene side chain was (*E*)-configured. The configuration at C(16) was derived from the well-known chemical shift of the MeO_2C group, which was basically the same as for akuammiline [14] and deacetylakuammiline [15]. On the basis of above analysis and by comparison with published values for deacetylakuammiline [14][15], the structure of **2** was thus established.

Compound **3**, obtained as a yellowish amorphous solid, was optically active ($[\alpha]_D^{24} = 48$ ($c = 0.23$, C_5H_5N)). The molecular formula was derived as $C_{22}H_{28}N_2O_4$ by positive-mode HR-ESI-MS (*m*/*z* 385.2122 ($[M+H]^+$; calc. 385.2127)). The IR spectrum of **3** showed typical bands at 3427 (OH), 2938 (C–H), 1734 (MeO_2C), and 1605 cm^{-1}

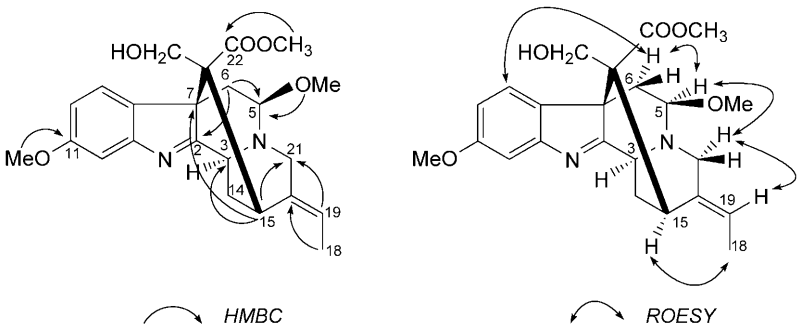

Fig. 2. *Key HMBC and ROESY correlations for* **2**

(C=C). The UV spectrum displayed characteristic absorptions at 225, 275, 280, and 291 nm, associated with an indole chromophore. The ^{1}H-NMR spectrum (*Table 1*) exhibited signals of four aromatic H-atoms at δ(H) 7.05 (*t*, $J=7.6$ Hz), 7.14 (*t*, $J=7.6$ Hz), 7.38 (*d*, $J=7.6$ Hz), and 7.39 (*d*, $J=7.6$ Hz). The MeO signal at δ(H) 3.62 belonged to a MeO_2C group, and the presence of an ethylidene side chain was evident [δ(H) 1.68 (*d*, $J=6.5$ Hz, Me(18)); 5.69 (*q*, $J=6.5$ Hz, H–C(19))]. These spectroscopic features were similar to those of isositsirikine-type alkaloids, except for an additional resonance due to a hydroxymethylene moiety at δ(H) 5.40 (*s*, CH_2(23)), whose position was assigned by its HMBC correlations with δ(C) 133.8 (C(2)) and 137.1 (C(13)).

The configuration of **3** was determined by a ROESY experiment (*Fig. 3*). Based on the cross-peaks between the pairs H–C(15)/H–C(18) and H–C(19)/H–C(21), the ethylidene side chain was assigned (*E*)-configuration. Further, the chemical shift of H–C(3) [δ(H) 4.04 (*d*, $J=10.1$ Hz)] was close to that of the corresponding H-atom in (16*S*,*E*)-isositsirikine, in which H_α–C(3) appears at δ(H) 3.90 [8].

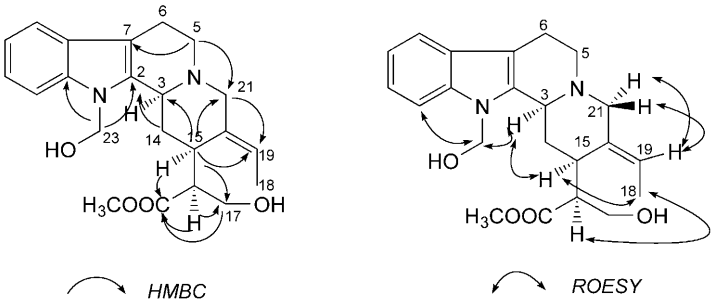

Fig. 3. *Key HMBC and ROESY correlations for* **3**

Experimental Part

General. All solvents were distilled before use. Column chromatography (CC) was performed on silica gel (100–200 mesh; *Qingdao Marine Chemical, Inc.*, China), silica gel *H* (10–40 μm; *Qingdao*), C_{18} reverse-phase (RP) silica gel (60 μm; *Merck*, Germany), and *Sephadex LH-20* (*Amersham Biosciences*, Sweden). Thin-layer chromatography (TLC) was performed on plates precoated with silica gel F_{254} (*Qingdao*); visualization under UV light and by spraying with *Dragendorff* reagent. UV Spectra: *Shi-*

madzu 210A double-beam spectrophotometer; λ_{max} (log ε) in nm. Optical rotations: *Horiba SEAP-300* spectropolarimeter. IR Spectra: *Bio-Rad FTS-135* spectrophotometer; in cm^{-1}. ^1H- and ^{13}C-NMR Spectra: *Bruker AM-400* spectrometer; δ in ppm, J in Hz. 2D-NMR Spectra: *Bruker DRX-500* apparatus. HR-ESI-MS: *VG AutoSpec-3000* mass spectrometer; in m/z.

Plant Material. The air-dried roots of *Rauvolfia yunnanensis* TSIANG were collected in Xishuangbanna, Yunnan Province, P. R. China, in October 2004. The plant was identified by Prof. *Hua Peng*, and a voucher specimen was deposited at the Kunming Institute of Botany, Yunnan, P. R. China.

Extraction and Isolation. The air-dried roots of *R. yunnanensis* (20 kg) were extracted with 95% EtOH (3×30 l) for 4, 3 and 3 h, resp. The EtOH extract was evaporated to dryness under reduced pressure, and the residue was suspended in H_2O. The pH of the suspension was adjusted to 3.0 by addition of 1% aq. HCl, and the suspension was extracted with $CHCl_3$. Then the pH of the aq. phase was adjusted to 10.5 by adding NaOH, and another extraction with $CHCl_3$ was carried out. The latter $CHCl_3$ extract contained the highly basic alkaloids as a crude material (100 g). These were further separated by initial CC (SiO_2 (200–300 mesh); $CHCl_3$/MeOH gradient): fractions *Fr. 1–Fr. 5. Fr. 1* (8.4 g) was re-subjected to CC (SiO_2; petroleum ether (PE)/AcOEt 4:1), which afforded ajmalicine (1.2 g) after recrystallization from PE/AcOEt 1:4. *Fr. 2* (1.6 g) was subjected to repeated CC (1. SiO_2; PE/AcOEt 3:1→1:4; 2. $CHCl_3$/MeOH 10:0.2) and prep. TLC (SiO_2; $CHCl_3$/MeOH 10:0.4) to afford reserpine (28 mg), ajmaline (25 mg), and yohimbine (80 mg). *Fr. 3* (11.4 g) was purified by CC (1. SiO_2, AcOEt/MeOH 10:0.2→10:6; 2. *Sephadex LH-20*, $CHCl_3$/MeOH 1:1) and then prep. TLC (SiO_2; $CHCl_3$/MeOH 10:1 and 9:1) to afford venoterpine (25 mg) and 19-epi-ajmalicine (30 mg). *Fr. 4* (8.4 g) was purified by CC (1. SiO_2, AcOEt/MeOH 10:1.5; 2. silica gel *H*; PE/AcOEt/Et_2NH 20:10:2→10:10:2) and then prep. TLC (SiO_2; $CHCl_3$/MeOH 10:1.2) to afford **1** (5 mg) and **2** (10 mg). *Fr. 5* was submitted to CC (1. SiO_2, $CHCl_3$/MeOH 10:0.4; 2. *RP-18*, MeOH) and then by prep. TLC (SiO_2; AcOEt/MeOH/Et_2NH 10:0.6:0.2) to afford **3** (45 mg) and (16*R*,19*E*)-isositsirikine (9 mg).

10-Methoxy-16-de(methoxycarbonyl)pagicerine (=*(15α,16*R,*19*E*)-10-Methoxy-17,22-epoxyvobasan-3-one*; **1**). Yellowish, amorphous solid. UV (MeOH): 209 (4.29), 322 (3.98). $[\alpha]_D^{24} = -97$ ($c = 0.58$, C_5H_5N). IR (KBr): 3425, 2925, 2854, 1626, 1522, 1459, 1291, 1250, 1217, 1166, 1107, 1026, 943, 858, 816, 610. ^1H- and ^{13}C-NMR: see *Tables 1* and *2*, resp. HR-ESI-MS: 353.1875 ($[M+H]^+$, $C_{21}H_{25}N_2O_3^+$; calc. 353.1865).

(5β)-17-O-*Deacetyl-5,11-dimethoxyakuammiline* (=*Methyl (5β,15α,16*R,*19*E*)-17-Hydroxy-5,11-dimethoxyakuammilan-16-carboxylate*; **2**). Yellowish, amorphous solid. UV (MeOH): 208 (4.24), 224 (4.20), 230 (4.21), 289 (3.53). $[\alpha]_D^{24} = -124$ ($c = 0.46$, C_5H_5N). IR (KBr): 3426, 2938, 1734, 1601, 1480, 1440, 1277, 1232, 1145, 1123, 1078, 1029, 932, 847, 769, 636. ^1H- and ^{13}C-NMR: see *Tables 1* and *2*, resp. HR-ESI-MS: 413.2069 ($[M+H]^+$, $C_{23}H_{29}N_2O_5^+$; calc. 413.2076).

*((16*S,*19*E*)-N^1-(Hydroxymethyl)isositsirikine* (=*Methyl (2*S*)-2-[(2*R,*3*E,*12b*S*)-3-Ethylidene-1,2,3,4,6,7,12,12b-octahydro-12-(hydroxymethyl)indolo[2,3-*a*]quinolizin-2-yl]-3-hydroxypropanoate*; **3**). Yellowish, amorphous solid. UV (MeOH): 225 (4.46), 275 (3.81), 280 (3.81), 291 (3.70), 347 (2.75), 363 (2.78). $[\alpha]_D^{24} = 48$ ($c = 0.23$, C_5H_5N). IR (KBr): 3427, 2923, 1729, 1630, 1464, 1342, 1194, 1034, 744, 562. ^1H- and ^{13}C-NMR: see *Tables 1* and *2*, resp. HR-ESI-MS: 385.2122 ($[M+H]^+$, $C_{22}H_{29}N_2O_4^+$; calc. 385.2127).

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