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TAXIFOLIN APIOSIDE AND DAVURICIIN M_1, A HYDROLYSABLE TANNIN FROM *ROSA DAVURICA**

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Key Word Index—*Rosa davurica*; Rosaceae; (+)-taxifolin-3-*O*-β-D-apio-D-furanoside; dihydroflavonol apioside; tannin; davuriciin M_1; ellagitannin.

Abstract—The structure of a dihydroflavonol glycoside from the roots of *Rosa davurica* has been characterized as (+)-taxifolin-3-*O*-β-D-apio-D-furanoside. A new hydrolysable tannin, davuriciin M_1, together with three known hydrolysable tannins, have also been isolated. The structure of new tannin was elucidated by spectroscopic and chemical methods.

INTRODUCTION

Rosa davurica Pall. is a shrub which is widely distributed in the northeast part of China, especially in Heilongjiang and Jilin Provinces. Its fruits, roots and flowers have been used in traditional Chinese medicine for the treatment of inflammation of the stomach and indigestion [1]. The fruit of this plant, which is rich in vitamin C, is now used as a health drink. The chemical constituents of this plant, however, have received little attention. In our continuing studies on rosaceous medicinal plants, we have examined the polyphenolic constituents of the roots, and isolated three known hydrolysable tannins, a new tannin named davuriciin M_1, and a new dihydroflavonol glycoside.

RESULTS AND DISCUSSION

The aqueous acetone extract of the roots of *R. davurica* afforded the flavonoid glycoside **1**, four hydrolysable tannins including a new tannin, davuriciin M_1, casuarictin (**3**) [2], 1,2,3,6-tetra-*O*-galloyl-β-D-glucose (**4**) [3], and 1,2,3,4,6-penta-*O*-galloyl-β-D-glucose (**5**) [4].

The flavonoid glycoside (**1**), $C_{20}H_{20}O_{11}$, gave UV absorptions [290 and 330 nm (sh)] characteristic of dihydroflavonols. Its ^{1}H NMR spectrum (acetone-d_6) exhibited two *meta*-coupled proton signals at δ5.93 and 5.96 (*d* each, $J=2$ Hz), signals of an ABX system at δ6.87 (*d*, $J=8.5$ Hz), 6.91 (*dd*, $J=2$, 8.5 Hz) and 7.07 (*d*, $J=2$ Hz), and also a strongly chelated hydroxyl proton signal (5-OH) at δ11.91. The aliphatic proton signals at δ5.15 and 4.66, which are in the *trans* relationship as revealed by their coupling constants ($J=11$ Hz), were assigned to H-2 and H-3 of dihydroflavonol, respectively. The ^{1}H–^{1}H shift correlation spectrum of **1** showed the presence of two methine protons [δ4.34 (*s*) and 3.79 (*d*, $J=4.5$ Hz)] and two methylene groups [δ4.15, 3.67 (1H each, *d*, $J=9.5$ Hz), and 3.74 and 3.67 (1H each, *dd*, $J=5.5$, 11 Hz)] in the sugar residue. Signals due to a primary, a secondary and a tertiary hydroxyl proton were present at δ3.85 (*t*, $J=5.5$ Hz), 4.26 (*d*, $J=4.5$ Hz) and 4.07 (*s*), and, as expected, all disappeared upon addition of D_2O. The first two were coupled with a methine proton at δ3.79, and methylene protons at δ3.74 and 3.67, which were collapsed into a singlet, and doublets, respectively, in the presence of D_2O. Acetylation of **1** gave the heptaacetate **1a** and the hexaacetate **1b**, verifying the presence of three aliphatic hydroxyl groups. Based on these data, compound **1** was presumed to be a taxifolin glycoside, the sugar of which was a pentose branched at C-3. A ^{1}H–^{13}C correlation spectrum permitted assignment of the respective carbons of taxifolin and the pentose residue, and the data of the latter coincided well with those reported for the apiosyl residue of glycosides [5] (Table 1). The CD spectrum of **1** showed a positive Cotton effect at 328 nm and a negative one at 293 nm, indicative of the 2(*R*),3(*R*) configuration of taxifolin [6] [7]. This structural assignment was confirmed by acid hydrolysis of **1** which yielded (+)-taxifolin (**2**) $[\alpha]_D+26°$ (Me_2CO–H_2O 1:1), and apiose.

Upon comparison of the ^{13}C NMR spectrum of **1** with that of **2**, it is observed that the C-3 signal of **1** is 2.6 ppm downfield from that of **2** [8], whereas the C-2 and C-4 signals of **1** are shifted upfield by 1.7 and 1.8 ppm, respectively (Table 1). The location of the apiose residue is thus determined to be at the C-3 position of **1**. The β-configuration of the glycosidic linkage of the apiosyl residue was determined as follows. The ^{1}H NMR spectrum of **1** showed sharp singlets for H-1″ and H-2″, indicating these protons to be *trans* on the furanose ring, and thus the glycosidic configuration to be β [9]. The chemical shift of the anomeric carbon (δ109.4) is also consistent with the value expected for the *trans* relationship between the glycosidic linkage at C-1″ and the adjacent hydroxyl group at C-2″, rather than the value (δ*ca* 103) for the *cis* analogue [10]. The difference ($\Delta[M]_D$ −192°) in the molecular rotation between **1**, $[M]_D-113°$, and its aglycone **2**, $[M]_D+79°$, is in accordance with that ($\Delta[M]_D-167°$) for methyl β-D-apio-D-furanoside [11].

* Part 6 in the series 'Tannins of Rosaceous Medicinal Plants'. For Part 5, see T. Yoshida, K. Tanaka, X.-M. Chen and Okuda, T. (1989) *Chem. Pharm. Bull.* **37**, 141.

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Table 1. ^{13}C NMR spectral data for compounds **1** and **2** (126 MHz, Me_2CO-d_6)

	C	1	2*	Apiosyl moiety of crocosmioside A†
Aglycone	2	83.0	84.7	
	3	75.8	73.2	
	4	196.1	197.9	
	5	167.7	168.1	
	6	97.0	97.0	
	7	165.0	164.8	
	8	95.9	95.6	
	9	163.6	164.0	
	10	102.2	101.3	
	1′	128.9	129.3	
	2′	115.4	115.6	
	3′	145.9	145.8	
	4′	146.7	146.6	
	5′	115.9	115.6	
	6′	120.5	120.4	
Apiosyl	1″	109.4		109.6
	2″	77.1		78.3
	3″	80.4		80.7
	4″	65.9		65.4
	5″	75.6		75.4

*Data from ref. [8] (MeOH-d_4).
†Data from ref. [5] (pyridine-d_5).

In the ROESY measurement of **1a**, appreciable NOE between H-2″ and the branching methine protons (H-4″), which supports the D-apio-D-furanoside structure, was observed. The other NOEs observed between protons of sugar and aglycone moieties are illustrated in conformation **1a**′. The structure of **1**, therefore, is (+)-taxifolin-3-*O*-β-D-apio-D-furanoside.

Although apiose is frequently encountered in nature as a sugar moiety of a variety of glycosides, e.g. flavone and flavonol glycosides [12], flavanone glycosides [13], coumarin glycosides [14], saponins [5] [15], and phenylpropanoid glycosides [16], etc., (+)-taxifolin-3-apioside (**1**) appears to be the first dihydroflavonol apioside.

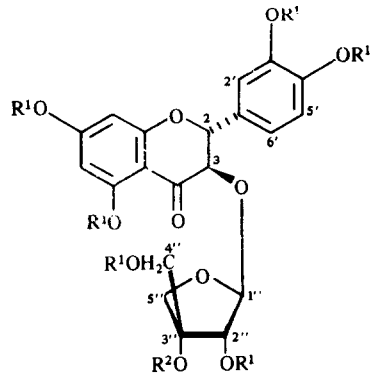
Davuriciin M_1 (**6**) on acid hydrolysis afforded, in addition to glucose, ellagic acid (**7**) and sanguisorbic acid dilactone (**8**) [17] which were identified by co-chromatography with authentic samples in reversed-phase HPLC, and further confirmed by characterization of the methylated derivatives **7a** and **8a** (^1H NMR). The ^1H NMR spectrum of **6** showed the presence of two hexahydroxydiphenoyl (HHDP) groups (δ6.63, 6.50, 6.33, 6.28, 1H each, *s*) and a sanguisorboyl group [δ7.29, 6.86 (1H each, *d*, $J = 2$ Hz), and 7.56 (1H, *s*)], of which the latter exists in a dilactone form. The chemical shifts and coupling pattern of the glucose proton signals of **6** showed close similarity to those of casuarictin (**3**), suggesting that the glucose residue is a fully acylated $^4C_1$ glucopyranose. The ^{13}C NMR spectrum of **6** exhibited seven ester carbonyl carbon signals, two of which resonate at higher field (δ156.6 and 159.9) than the others, and are assigned to those of the lactonic carbonyl groups.

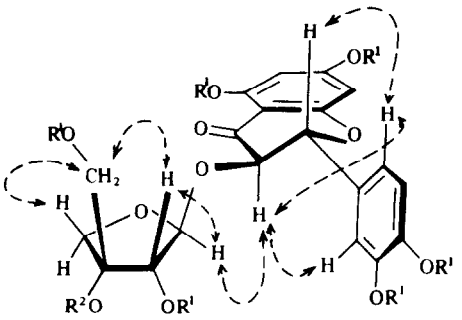
The location of the sanguisorboyl group at C-1 was unequivocally established by the ^1H–^{13}C long-range ($J_{CH} = 7$ Hz) 2D spectrum. The carbonyl carbon signal at δ164.7, which couples with both *meta*-coupled protons (H_A and H_B) of the sanguisorboyl group through a three-bond coupling, is also coupled with the anomeric proton at δ6.04. The β-configuration at the anomeric centre is evidenced by the coupling constant ($J = 8.5$ Hz) of H-1. The CD spectrum of **6** showed a positive Cotton effect at 238 nm and a negative one at 265 nm, which are indicative of the (*S*)-configuration of both HHDP groups [18]. Therefore, the structure of davuriciin M_1 is 1-*O*-sanguisorboyl dilactone-2,3; 4,6-di-*O*-(*S*)-HHDP-β-D-glucose (**6**).

EXPERIMENTAL

General. NMR spectra were recorded at 500 MHz for ^1H and 126 MHz for ^{13}C. Chemical shifts are given in δ relative to Me_2CO-d_6, and converted relative to TMS by adding 2.04 (^1H) and 29.8 (^{13}C). Kieselgel PF_{254} was used for TLC and PTLC.

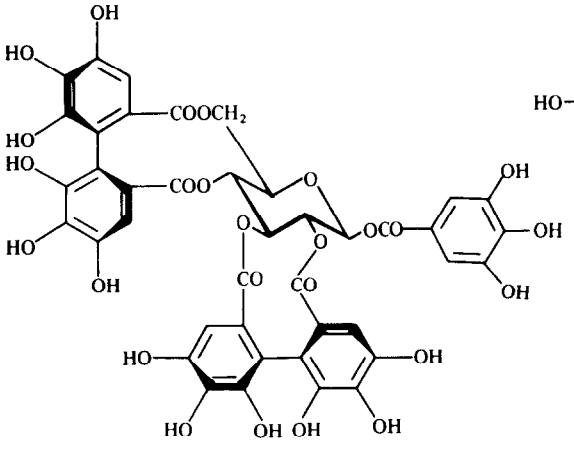
Isolation of polyphenols. Dried roots (520 g) of *R. davurica*, collected at Harbin, Heilongjiang, China, were chopped into fine pieces, extracted with 70% aq. Me_2CO, and concd. After removal of the ppt. deposited upon concentration, the aq. soln was subjected to CC over Diaion HP-20 (5 i.d. × 48 cm) (Mitsubishi Chemical Industries Ltd, Japan) using H_2O containing increasing amounts of MeOH as eluant. The residue (5.2 g) obtained from the 40% aq. MeOH eluant was rechromatographed over Toyopearl HW-40 (2.2 i.d. × 38 cm) (coarse grade; TOSOH, Japan) developed with 60% aq. MeOH → 70% aq. MeOH → MeOH–H_2O–Me_2CO (7:2:1), and 15 g portions were collected. Fractions 51–62 (60% aq. MeOH) gave 1,2,3,6-tetra-*O*-galloyl-β-D-glucose (**4**) (13 mg) after purification by CC on Toyopearl HW-40 (fine grade) with 50% aq MeOH. Fractions 278–283 and 318–360 (MeOH–H_2O–Me_2CO, 7:2:1) were similarly purified separately by CC on Toyopearl HW-40 (fine grade) using 70% aq. MeOH → MeOH–H_2O–Me_2CO (7:2:1) to give casuarictin (**3**) (30 mg), and davuriciin M_1 (**6**) (21 mg), respectively. The 50% MeOH eluate from CC on Diaion HP-20 was



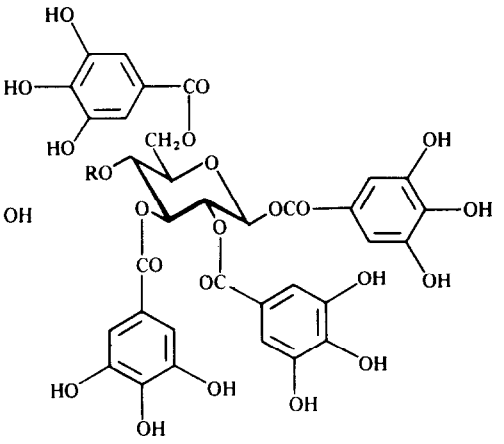


1a' $R^1 = R^2 = Ac$

1 $R^1 = R^2 = H$
1a $R^1 = R^2 = Ac$
1b $R^1 = Ac$, $R^2 = H$



3



4 R = H

5 R = —CO— (OH, OH, OH)

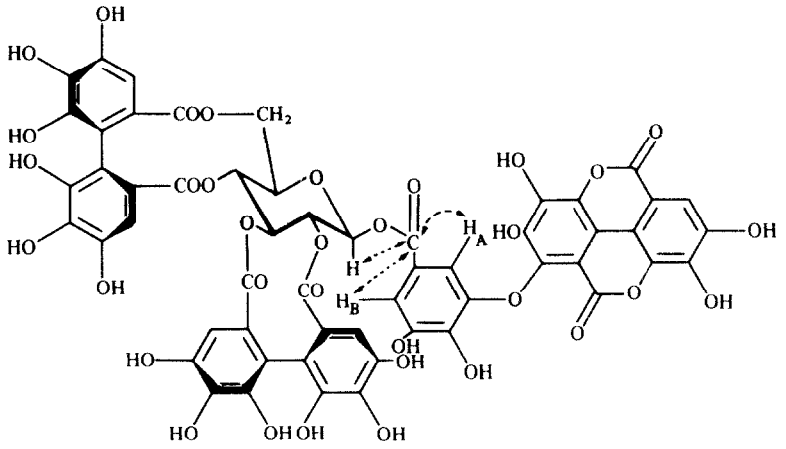
Scheme 1.

also further fractionated by CC on Toyopearl HW-40 (fine grade) with 60% aq. MeOH → MeOH → MeOH–H_2O–Me_2CO (7:2:1) to give, in order of elution, (+)-taxifolin-3-*O*-*β*-D-apio-D-furanoside (**1**) (667 mg), 1,2,3,4,6-penta-*O*-galloyl-*β*-D-glucose (**5**) (20 mg) and davuriciin M_1 (**6**) (77 mg).

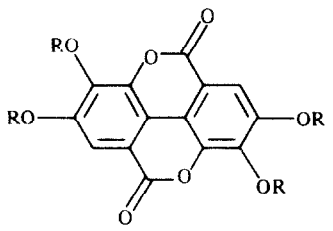
(+)-*Taxifolin*-3-O-*β*-D-*apio*-D-*furanoside* (**1**). White amorphous powder, $[\alpha]_D - 26°$ (Me_2CO–H_2O 1:1; *c* 1.1); UV λ_{max}^{MeOH} nm (log ε): 290 (4.26), 330 (sh) (3.63); FABMS (negative): *m/z* 435 $[M-H]^-$ ($C_{20}H_{20}O_{11} = 436$); ^1H NMR (Me_2CO–d_6): δ3.67 (1H, *dd*, *J* = 5.5, 11 Hz), 3.74 (1H, *dd*, *J* = 5.5, 11 Hz) (H-4″), 3.67 (1H, *d*, *J* = 9.5 Hz), 4.15 (1H, *d*, *J* = 9.5 Hz) (H-5″), 3.85 (1H, *br t*, *J* = 5.5 Hz, 4″-OH), 3.79 (1H, *d*, *J* = 4.5 Hz, H-2″), 4.26 (1H, *d*, *J* = 4.5 Hz, 2″-OH), 4.07 (1H, *s*, 3″-OH), 4.34 (1H, *s*, H-1″), 4.66 (1H, *d*, *J* = 11 Hz, H-3), 5.15 (1H, *d*, *J* = 11 Hz, H-2), 5.93 (1H, *d*, *J* = 2 Hz, H-8), 5.96 (1H, *d*, *J* = 2 Hz, H-6), 6.87 (1H, *d*, *J* = 8.5 Hz, H-5′), 6.91 (1H, *dd*, *J* = 2, 8.5 Hz, H-6′), 7.07 (1H, *d*, *J* = 2 Hz, H-2′), 11.91 (1H, *s*, 5-OH); (Me_2CO-d_6–D_2O): δ3.67 (1H, *d*, *J* = 11.5 Hz), 3.74 (1H, *d*, *J* = 11.5 Hz), (H-4″), 3.67 (1H, *d*, *J* = 9.5 Hz), 4.15 (1H, *d*, *J* = 9.5 Hz) (H-5″), 3.79 (1H, *br s*, H-2″), 4.33 (1H, *s*, H-1″); ^{13}C NMR: see Table 1; CD (MeOH; *c* 0.01); $[\theta]_{328} + 8 \times 10^3$, $[\theta]_{293} - 29 \times 10^3$.

Acetylation of **1**. Compound **1** (10 mg) was acetylated in the usual way to give a mixture of a heptaacetate (**1a**) (2.5 mg) and a hexaacetate (**1b**) (0.4 mg), which was separated by prep. TLC (silica gel, C_6H_6–Me_2CO 4:1). *Heptaacetate* (**1a**). White amorphous powder, $[\alpha]_D + 13°$ (Me_2CO; *c* 1.9); ^1H NMR ($CDCl_3$): δ1.97, 1.99, 2.11, 2.29, 2.296, 2.30, 2.38 (3H each, *s*, OAc), 4.09 (1H, *d*, *J* = 10.5 Hz), 4.44 (1H, *d*, *J* = 10.5 Hz) (H-5″), 4.18 (1H, *s*, H-1″), 4.44 (1H, *d*, *J* = 12 Hz), 5.28 (1H, *d*, *J* = 12 Hz) (H-4″), 4.78 (1H, *d*, *J* = 12.5 Hz, H-3), 5.02 (1H, *d*, *J* = 12.5 Hz, H-2), 5.26 (1H, *s*, H-2″), 6.58 (1H, *d*, *J* = 2 Hz, H-6), 6.77 (1H, *d*, *J* = 2 Hz, H-8), 7.27 (1H, *d*, *J* = 8.5 Hz, H-5′), 7.37 (1H, *d*, *J* = 2 Hz, H-2′), 7.42 (1H, *dd*, *J* = 2, 8.5 Hz, H-6′).

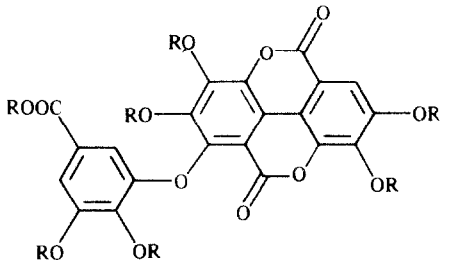
Hexaacetate (**1b**). White amorphous powder, $[\alpha]_D - 18°$ (Me_2CO; *c* 1.3); ^1H NMR (Me_2CO-d_6): δ1.98, 2.02, 2.276, 2.28, 2.283, 2.30 (3H each, *s*, OAc), 3.73 (1H, *d*, *J* = 10 Hz), 4.24 (1H, *d*,



6



7 R = H
7a R = Me



8 R = H
8a R = Me

Scheme 2.

$J = 10$ Hz) (H-5″), 4.20 (1H, *d*, $J = 11.5$ Hz), 4.34 (1H, *d*, $J = 11.5$ Hz) (H-4″), 4.24 (1H, *s*, H-1″), 4.89 (1H, *s*, H-2″), 4.71 (1H, *d*, $J = 11.5$ Hz, H-3), 5.50 (1H, *d*, $J = 11.5$ Hz, H-2), 6.65 (1H, *d*, $J = 2$ Hz, H-6), 6.82 (1H, *d*, $J = 2$ Hz, H-8), 7.33 (1H, *d*, $J = 8.5$ Hz, H-5′), 7.54 (1H, *d*, $J = 2$ Hz, H-2′), 7.56 (1H, *dd*, $J = 2$, 8.5 Hz, H-6′).

Hydrolysis of **1**. A soln of **1** (10 mg) in 1% H_2SO_4 (0.5 ml) was refluxed for 1 hr. After cooling, the reaction mixture was extracted with EtOAc. The EtOAc layer, after drying with $MgSO_4$, was concd to dryness to give (+)-taxifolin (**2**) (2.5 mg), $[\alpha]_D + 26°$ (MeOH; *c* 0.5). The aq. layer was neutralized with Amberlite IRC-410 (OH form), and the resin was filtered off. The filtrate was evapd to dryness. The residue was shown by GC (2% OV-1, column temp. 170°) to contain D-apiose (R_t 2.8 and 3.4 min) which was identified by co-chromatography with an authentic sample obtained from apiin {7-*O*-[β-D-apio-D-furanosyl-(1 → 2)-β-D-glucopyranosyl]apigenin} [19].

Davuriciin M_1 (**6**). Off-white amorphous powder, $[\alpha]_D - 58°$ (MeOH; *c* 1.0); UV λ_{max}^{MeOH} nm (log ε): 220 (4.88), 254 (4.73); FABMS (negative) *m/z*: 1235 $[M-H]^-$ ($C_{55}H_{32}O_{34}$ 1236); ^{1}H NMR (Me_2CO-d_6): δ7.56 (1H, *s*), 7.29 (1H, *d*, $J = 2$ Hz), 6.86 (1H, *d*, $J = 2$ Hz) (sanguisorboyl), 6.63, 6.50, 6.33, 6.28 (1H each, *s*) (HHDP), 6.04 (1H, *d*, $J = 8.5$ Hz, H-1), 5.04 (1H, *t*, $J = 8.5$ Hz, H-2), 5.33 (1H, *dd*, $J = 8.5$, 10 Hz, H-3), 5.08 (1H, *t*, $J = 10$ Hz, H-4), 4.38 (1H, *ddd*, $J = 1.5$, 7, 10 Hz, H-5), 5.27 (1H, *dd*, $J = 7$, 13 Hz, H-6), 3.78 (1H, *dd*, $J = 1.5$, 13 Hz, H-6′); ^{13}C NMR (Me_2CO-d_6): δ62.8 (C-6), 69.9 (C-4), 73.2 (C-5), 75.6 (C-2), 77.0 (C-3), 92.1 (C-1), 169.2, 168.4, 168.0, 167.8, 164.7, 159.9, 156.6 (carbonyl); CD (MeOH): $[\theta]_{238} + 12.2 \times 10^4$ $[\theta]_{265} - 7.3 \times 10^4$; $[\theta]_{283} + 0.4 \times 10^4$; $[\theta]_{310} - 0.9 \times 10^4$.

Acid hydrolysis of **6**. Davuriciin M_1 (**6**) (10 mg) was hydrolysed with 1% H_2SO_4 (1 ml) under reflux for 9 hr. After removal of the precipitate by centrifugation, the supernatant was neutralized with Amberlite IRC-410 (OH), and the resin filtered off. The filtrate gave glucose which was identified by GC (2% OV-1, column temp. 170°) after trimethylsilylation. The ppt. was methylated with CH_2N_2 to afford, after prep. TLC (silica gel, C_6H_6–Me_2CO, 15:1), tetra-*O*-methylellagic acid (**7a**) (1 mg) and methyl hexa-*O*-methylsanguisorbate dilactone (**8a**) (1.2 mg) [^{1}H NMR (Me_2CO-d_6): δ7.72 (1H, *s*), 7.35 (1H, *d*, $J = 2$ Hz), 6.80 (1H, *d*, $J = 2$ Hz), 3.78, 3.88, 3.96, 4.05, 4.12, 4.19 and 4.29 (3H each, *s*)].

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