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ACYLATED PELARGONIDIN 3-SOPHOROSIDE-5-GLUCOSIDES FROM THE FLOWERS OF THE JAPANESE MORNING GLORY CULTIVAR 'VIOLET'

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Abstract — Two novel acylated anthocyanins were isolated from the red-purple flowers of Japanese morning glory 'Violet' (or 'Murasaki') along with three known pigments. These novel pigments were determined to be pelargonidin 3-*O*-[2-*O*-(6-*O*-(*trans*-caffeoyl)-β-D-glucopyranosyl)-β-D-glucopyranoside]-5-*O*-β-D-glucopyranoside (**1**), and pelargonidin 3-*O*-[2-*O*-(6-*O*-(*trans*-*p*-coumaroyl)-β-D-glucopyranosyl)-β-D-glucopyranoside]-5-*O*-β-D-glucopyranoside (**2**). Three known pigments were also identified to be *Pharbitis* Red anthocyanin 1, and *Ipomoea* Red anthocyanins 1 and 4.

During the course of our investigation on the flower color variation of the Japanese morning glory (*Pharbitis nil* or *Ipomoea nil*) as well as the common morning glory (*Ipomoea purpurea*), we have found the presence of ten acylated pelargonidin 3-sophoroside-5-glucosides in the red – purple flowers of these plants.[1,2] All of these pigments were elucidated to be substituted with the complicated caffeoylsophorose residues at 3-OH of pelargonidin. As part of our continuing effort to determine the structures, and study the chemotaxonomy and biology of novel acylated anthocyanins, we have isolated two new acylated pelargonidin 3-sophoroside-5-glucosides together with three known acylated pelargonidin glycosides in

red-purple cultivar 'Violet' or 'Murasaki' obtained from Marutane Co. Ltd., Kyoto. The cultivar 'Violet' has been used in most physiological studies of flowering in *P. nil.*[3] In this communication, we wish to report the structure determination of these two novel pigments. For the sake of convenience, we named these two new pigments as *Pharbitis* Red anthocyanins 4 and 7 (PRA-4 and –7).

Fresh red-purple flowers (500 g) of *P. nil* 'Violet' were extracted with 5% AcOH. By the analysis of HPLC,[5] 13 anthocyanin peaks were observed in this extract. Among these 13 peaks,[5] four major peaks were found to correspond to *Pharbitis* Red anthocyanins 2,3,5, and pelargonidin 3-sophoroside-5-glucoside, previously isolated by us.[1] Therefore, we further investigated the isolation and structure determination of anthocyanins from the five minor peaks of this plant as follows.

The concentrated extract was purified by Dianion HP-20 column chromatography with 1% AcOH and 5% AcOH-MeOH, and then by paper chromatography with BAW (*n*-BuOH/AcOH/H_2O, 4:1:5, v/v) and 15% AcOH. The fractions containing five anthocyanins were further purified by preparative ODS-HPLC,[5] and precipitated with MeOH-Et_2O (containing 1% TFA) to afford pure pigments; pigment A (**1**) (*Pharbitis* Red anthocyanin 4)[6] (*ca.* 50 mg), pigment B (**2**) (*Pharbitis* Red anthocyanin 7)[7] (*ca.* 11 mg), pigment C (**3**) (*Pharbitis* Red anthocyanin 1)[1,8] (*ca.* 30 mg), pigment D (**4**) (*Ipomoea* Red anthocyanin 3)[2,9] (*ca.* 40 mg), and pigment E (**5**) (*Ipomoea* Red anthocyanin 1)[2,10] (*ca.* 10 mg).

By acid hydrolysis, pigments A – E (**1 – 5**) gave pelargonidin, glucose and caffeic acid, where exceptionally pigment B (**2**) gave *p*-coumaric acid instead of caffeic acid as the acyl moiety. The deacylanthocyanin of these five pigments A – E (**1 - 5**) was determined to be pelargonidin 3-sophoroside-5-glucoside by direct comparison of TLC and HPLC with an authentic sample of deacyl *Pharbitis* Red anthocyanins.[1] The FAB-MS spectra of pigments A – E (**1 – 5**) gave their molecular ions [M^+] at 919, 903, 919, 1081, and 1567 *m/z*, respectively, in good agreement with their mass calculated for $C_{42}H_{47}O_{23}$ as pigment A (**1**), $C_{42}H_{47}O_{22}$ as pigment B (**2**), $C_{42}H_{47}O_{23}$ as pigment C (**3**), $C_{51}H_{53}O_{26}$ as pigment D (**4**), and $C_{72}H_{79}O_{39}$ as pigment E (**5**). In comparison with authentic samples of *Pharbitis* Red anthocyanins[1] and *Ipomoea* Red anthocyanins,[2] it was revealed that three pigments C – E (**3 – 5**) were structurally known anthocyanins, however, this is the first report for the occurrence of pigments D (**4**) and E (**5**) in *P. nil.* These three pigments were identified to be pelargonidin 3-*O*-[2-*O*-(β-D-glucosyl)-6-*O*-(caffeoyl)-β-D-glucoside]-5-*O*-β-D-glucoside (*Pharbitis* Red anthocyanin 1), as pigment C (**3**),[8] pelargonidin 3-*O*-[2-*O*-(6-

O-caffeoyl-β-D-glucosyl)-6-*O*-(caffeoyl)-β-D-glucoside]-5-*O*-β-D-glucoside (*Ipomoea* Red anthocyanin 4), as pigment D (**4**),[9] and pelargonidin 3-*O*-[2-*O*-(6-*O*-(3-*O*-β-D-glucosylcaffeoyl)-β-D-glucosyl)-6-*O*-(4-*O*-(6-*O*-caffeoyl-β-D-glucosyl)caffeoyl)-β-D-glucoside]-5-*O*-β-D-glucoside (*Ipomoea* Red anthocyanin 1), as pigment E (**5**),[10] by the analyses of TLC and HPLC. These structures were also confirmed by the analyses of ^{1}H and ^{13}C NMR spectra.[8,10]

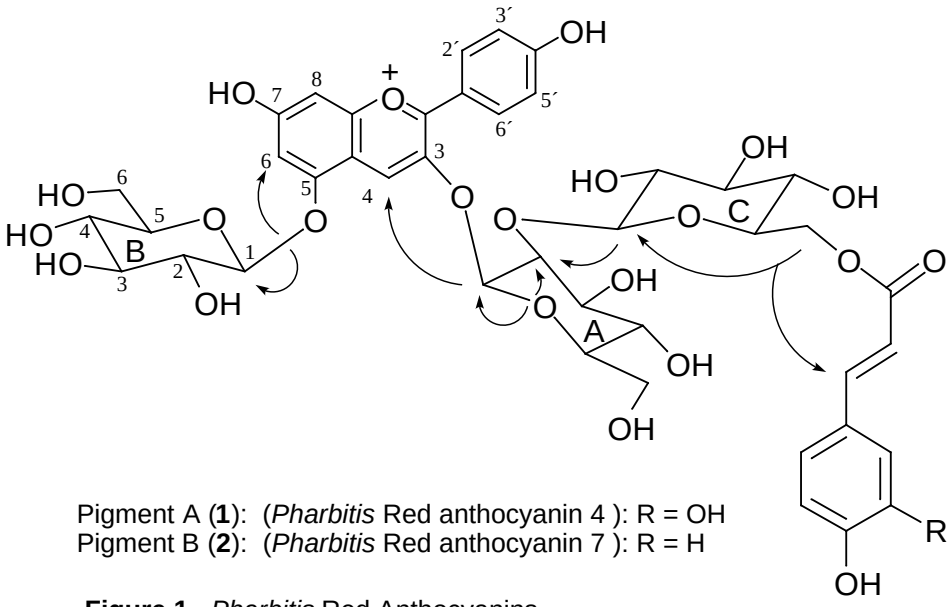

Figure 1 *Pharbitis* Red Anthocyanins.
Observed NOE's are indicated by arrows.

Pigment A (**1**) (*Pharbitis* Red anthocyanin 4). ^{1}H NMR and and FAB-MS of pigment A (**1**) (m/z 919) showed the presence of each one molecule of pelargonidin and caffeic acid, and three molecules of glucose. Ten aromatic proton signals of pelargonidin and caffeic acid were assigned by analysis of its $^{1}H - {}^{1}H$ COSY spectrum as shown in Table 1. Two olefinic proton signals of caffeic acid in this pigment had large coupling constants (J = 15.6 Hz), indicating caffeic acid to have *trans* configuration. Regarding the sugar moieties of this pigment, the signals of three anomeric protons were observed at δ 5.52 (d, J = 7.6 Hz, Glc A), δ 5.18 (d, J = 7.6 Hz, Glc B), and δ 4.96 (d, J = 7.9 Hz, Glc C), and the assigned glucose protons had coupling constants (J = 7.6 – 10.4 Hz). These data strongly supported that these glucose residues must be β-D-glucopyranose forms (Figure 1). Moreover, two characteristic proton signals shifted to the lower magnetic field at δ 4.14 (2H, m, H-6a and –6b) were assigned to the methylene (C-6) of Glc C by

Table 1 NMR spectral data for anthocyanins of *Pharbitis nil* cv. 'Violet'.*

	Pigment A (**1**) (*Pharbitis* Red anthocyanin 4)		Pigment B (**2**) (*Pharbitis* Red anthocyanin 7)
	δ C	δ H	δ H
Pelargonidin			
2	165.3		
3	144.2		
4	135.2	9.03 s	9.04 s
5	155.1		
6	104.1	7.03 d (1.5)	7.01 br s
7	168.0		
8	96.2	7.17 d (1.5)	7.16 br s
9	162.7		
10	111.8		
1'	119.1		
2'	135.2	8.69 d (9.2)	8.69 d (9.2)
3'	117.0	7.16 d (9.2)	7.16 d (9.2)
4'	155.5		
5'	117.0	7.16 d (9.2)	7.16 d (9.2)
6'	135.2	8.69 d (9.2)	8.69 d (9.2)
Cinnamic acid			
1	125.4		
2	115.0	6.96 d (1.8)	7.41 d (8.6)
3	145.5		6.82 d (8.6)
4	148.4		
5	115.8	6.79 d (8.2)	6.82 d (8.6)
6	121.1	6.77 dd (1.8, 8.2)	7.41 d (8.6)
α	145.1	7.31 d (15.6)	7.37 d (15.9)
β	113.6	6.07 d (15.6)	6.15 d (15.9)
C=O	166.4		
Glucose A			
1	100.1	5.52	5.50
2	79.8	3.99	3.99
3	76.7	3.70	3.69
4	77.4	3.30	3.55
5	69.4	3.38	3.36
6a, b	60.5	3.50-3.60, 3.77	3.51-3.58, 3.76
Glucose B			
1	101.2	5.18	5.16
2	73.0	3.53	3.53
3	75.9	3.41	3.43
4	69.6	3.30	3.30
5	77.4	3.50-3.60	3.51-3.58
6a, b	60.5	3.50-3.60, 3.80	3.51-3.58, 3.80
Glucose C			
1	103.5	4.96	4.95
2	74.3	3.12	3.13
3	74.0	3.32-3.35	3.30-3.38
4	76.3	3.32-3.35	3.30-3.38
5	69.5	3.32-3.35	3.30-3.38
6a, b	62.8	4.14, 4.14	4.16, 4.16

* ^{1}H NMR (500 MHz) (CF_3CO_2D-DMSO-d_6, 1:9), at 25 °C, an internal standard of TMS;
^{13}C NMR (125.65 MHz) (CF_3CO_2D-DMSO-d_6, 1:9), at 25 °C, an internal standard of TMS.
Coupling constants (*J* in Hz) in paretheses.

the analysis of 2D COSY spectrum, and also confirmed by negative NOE difference (DIFNOE) spectral measurement.[11] Therefore, Glc C was determined to be acylated at OH-6 with caffeic acid. The linkages of pelargonidin with sugar units were also confirmed based on the DIFNOE spectral study providing the following results. Glc A was bonded at OH-3 of pelargonidin, Glc B at OH-5 of pelargonidin, and Glc C at OH-2 of Glc A. (Figure 1) Thus, pigment A (**1**) is pelargonidin 3-*O*-[2-*O*-(6-*O*-(*trans*-caffeoyl)-β-D-glucopyranosyl)-β-D-glucopyranoside]-5-*O*-β-D-glucopyranoside, which is a new caffeoyl pelargonidin glycoside.[12,13] Its structure was further confirmed by the analyses of ^{13}C NMR, HMQC and HMBC spectra. (Table 1)

Pigment B (**2**) (*Pharbitis* Red anthocyanin 7). ^{1}H NMR and FAB-MS of pigment B (**2**) (*m/z* 903) showed the presence of each one molecule of pelargonidin and *p*-coumaric acid, and three molecules of glucose. By analysis of its ^{1}H – ^{1}H COSY spectrum, seven proton signals of pelargonidin and six proton signals of *p*-coumaric acid were assigned as shown in Table 1. A pair of doublet signal (δ 6.15 and 7.37) with large coupling constants (J = 15.9 Hz) indicated the presence of *trans*-olefinic protons in *p*-coumaric acid. The proton signals of three glucose units were observed in the region of δ 5.50 – 3.13, and their vicinal coupling constants were J = 7.6 – 10.4 Hz. Moreover, the chemical shifts of three anomeric protons appeared at δ 5.50 (d, J = 7.6 Hz, Glc A), δ 5.16 (d, J = 7.9 Hz, Glc B), and δ 4.95 (d, J = 7.9 Hz, Glc C), suggesting all the glucose units to be β-D-glucopyranoside. Two characteristic proton signals at δ 4.16 (2H, m, H-6a and –6b) were assigned to the methylene of Glc C by ^{1}H – ^{1}H COSY and DIFNOE spectra, indicating *p*-coumaric acid to be attached to OH-6 of Glc C. Thus, the structure of pigment B (**2**) must be pelargonidin 3-*O*-[2-*O*-(6-*O*-(*trans*-*p*-coumaroyl)-β-D-glucopyranosyl)-β-D-glucopyranoside]-5-*O*-β-D-glucopyranoside, which is a new anthocyanin.[12,13]

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5. HPLC was run on Inertsil ODS-2 column (4.6×250 mm for analysis, and 20×250 mm for prep.) at 35°C, with a flow rate of 0.8 mL/min^{-1} and monitoring at 520 nm. Solvent systems for analysis were as follows: linear gradient elution for 60 min from 25 – 85% solvent B (1.5% H_3PO_4, 20% AcOH, 25% MeCN) in solvemt A (1.5% H_3PO_4). The relative frequency of pigment occurrence in the flower extract by HPLC was 14.8% (pelargonidin 3-sophoroside-5-glucoside), 0.9% (unknown pigment), 14.9% (*Pharbitis* Red anthocyanin 2), 11.6% [pigment A (**1**)], 4.7% [pigment B (**2**)], 11.8% (PRA-3), 6.0% [pigment C (**3**)], 6.7% [pigment D (**4**)], 13.6% (PRA-5), 2.1% [pigment E (**5**)], 1.0% (unknown pigment), 1.8% (unknown pigment), 2.7% (unknown pigment).

6. Pigment A (**1**) (*Pharbitis* Red anthocyanin 4): UVλmax (0.1% HCl-MeOH) 505, 330, 285 nm, Eacyl/Emax = 0.64; HPLC Rt (min) 21.5.

7. Pigment B (**2**) (*Pharbitis* Red anthocyanin 7): UVλmax (0.1% HCl-MeOH) 504, 319, 284 nm, Eacyl/Emax = 0.64; HPLC Rt (min) 27.1.

8. Pigment C (**3**) (*Pharbitis* Red anthocyanin 1): UVλmax (0.1% HCl-MeOH) 509, 331, 288 nm, Eacyl/Emax = 0.56; HPLC Rt (min) 33.7. ^{1}H NMR (500 MHz)(CF_3CO_2D-DMSO-d_6, 1:9): δ pelargonidin: 8.91 (1H, s, H-4), 6.99 (1H, d, *J* = 2.1 Hz, H-6), 7.14 (1H, d, *J* = 2.1 Hz, H-8), 8.64 (2H, d, *J* = 9.2 Hz, H-2', 6'), 7.15 (2H, d, *J* = 9.2 Hz, H-3', 5'); caffeic acid: 7.06 (1H, d, *J* = 1.5 Hz, H-2), 6.80 (1H, d, *J* = 8.2 Hz, H-5), 6.91 (1H, dd, *J* = 1.5 and 8.2 Hz, H-6), 7.36 (1H, d, *J* = 15.9 Hz, H-α), 6.20 (1H, d, *J* = 15.9 Hz, H-β); Glc A: 5.69 (H-1), 4.11 (H-2), 3.79 (H-3), 3.41 (H-4), 4.00 (H-5), 4.37 (H-6a), 4.47 (H-6b); Glc B: 5.15 (H-1), 3.55 (H-2), 3.37 (H-3), 3.31 (H-4), 3.41 (H-5), 3.59 (H-6a), 3.85 (H-6b); Glc C: 4.78 (H-1), 3.06 (H-2), 3.20 (H-3), 3.11 (H-4), 2.94 (H-5), 3.33 (H-6a), 3.33 (H-6b).

9. Pigment D (**4**) (*Pharbitis* Red anthocyanin 3): UVλmax (0.1% HCl-MeOH) 510, 329, 289 nm, Eacyl/Emax = 0.94; HPLC Rt (min) 34.2. ^{1}H NMR (500 MHz)(CF_3CO_2D-DMSO-d_6, 1:9): δ pelargonidin: 8.95 (1H, s, H-4), 7.00 (1H, d, *J* = 1.8 Hz, H-6), 7.04 (1H, d, *J* = 1.8 Hz, H-8), 8.59 (2H, d, *J* = 9.2 Hz, H-2', 6'), 7.11 (2H, d, *J* = 9.2 Hz, H-3', 5'); caffeic acid: 6.98 (1H, d, *J* = 1.8 Hz, H-2), 6.80 (1H, d, *J* = 8.2 Hz, H-5), 6.88 (1H, dd, *J* = 1.8 and 8.2 Hz, H-6), 7.35 (1H, d, *J* = 15.9 Hz, H-α), 6.16 (1H, d, *J* = 15.9 Hz, H-β); caffeic acid: 6.91 (1H, d, *J* = 1.8 Hz,

H-2), 6.77 (1H, d, *J* = 8.2 Hz, H-5), 6.82 (1H, dd, *J* = 1.8 and 8.2 Hz, H-6), 7.23 (1H, d, *J* = 15.9 Hz, H-α), 5.96 (1H, d, *J* = 15.9 Hz, H-β); Glc A: 5.62 (H-1), 4.01 (H-2), 3.77 (H-3), 3.49 (H-4), 3.90 (H-5), 4.32 (H-6a), 4.45 (H-6b); Glc B: 5.14 (H-1), 3.52 (H-2), 3.44 (H-3), 3.30 (H-4), 3.58 (H-5), 3.57 (H-6a), 3.84 (H-6b); Glc C: 4.86 (H-1), 3.19 (H-2), 3.32 (H-3, -4, -5), 4.11 (H-6a, H-6b); ^{13}C NMR (125.65 MHz) (CF_3CO_2D-DMSO-d_6, 1:9): δ pelargonidin: 165.5 (C-2), 144.0 (C-3), 135.3 (C-4), 155.4 (C-5), 105.0 (C-6), 168.4 (C-7), 96.7 (C-8), 162.9 (C-9), 111.9 (C-10), 119.1 (C-1'), 135.1 (C-2'), 117.1 (C-3'), 155.6 (C-4'), 117.1 (C-5'), 135.2 (C-6'); caffeic acid: 125.6 (C-1), 115.3 (C-2), 145.6 (C-3), 148.5 (C-4), 116.0 (C-5), 121.4 (C-6), 145.6 (C-α), 114.1 (C-β), 166.4 (C=O); caffeic acid: 125.6 (C-1), 115.1 (C-2), 145.6 (C-3), 148.5 (C-4), 115.9 (C-5), 121.3 (C-6), 145.2 (C-α), 113.7 (C-β), 166.6 (C=O); Glc A: 100.2 (C-1), 81.0 (C-2), 76.1 (C-3), 69.8 (C-4), 73.4 (C-5), 63.0 (C-6); Glc B: 102.0 (C-1), 77.8 (C-2), 76.4 (C-3), 74.3 (C-4), 72.5 (C-5), 61.0 (C-6); Glc C: 104.2 (C-1), 74.6 (C-2), 69.9 (C-3), 76.4 (C-4), 69.9 (C-5), 63.0 (C-6).

10. Pigment E (**5**) (*Pharbitis* Red anthocyanin 1): UVλmax (0.1% HCl-MeOH) 512, 319, 287 nm, Eacyl/Emax = 1.06; HPLC Rt (min) 37.2. ^{1}H NMR (500 MHz)(CF_3CO_2D-DMSO-d_6, 1:9): δ pelargonidin: 8.95 (1H, s, H-4), 7.01 (1H, d, *J* = 1.8 Hz, H-6), 7.05 (1H, d, *J* = 1.8 Hz, H-8), 8.59 (2H, d, *J* = 9.1 Hz, H-2', 6'), 7.10 (2H, d, *J* = 9.1 Hz, H-3', 5'); caffeic acid: 7.51 (1H, d, *J* = 1.8 Hz, H-2), 7.12 (1H, d, *J* = 8.4 Hz, H-5), 7.20 (1H, dd, *J* = 1.8 and 8.4 Hz, H-6), 7.55 (1H, d, *J* = 15.9 Hz, H-α), 6.45 (1H, d, *J* = 15.9 Hz, H-β); caffeic acid: 6.92 (1H, d, *J* = 1.8 Hz, H-2), 6.77 (1H, d, *J* = 8.1 Hz, H-5), 6.82 (1H, dd, *J* = 1.8 and 8.1 Hz, H-6), 7.24 (1H, d, *J* = 15.7 Hz, H-α), 5.96 (1H, d, *J* = 15.7 Hz, H-β); caffeic acid: 7.10 (1H, br d, H-2), 6.84 (1H, d, *J* = 8.3 Hz, H-5), 6.94 (1H, m, H-6), 7.38 (1H, d, *J* = 16.0 Hz, H-α), 6.27 (1H, d, *J* = 16.0 Hz, H-β); Glc A: 5.62 (H-1), 4.01 (H-2), 3.77 (H-3), 3.49 (H-4), 3.92 (H-5), 4.33 (H-6a), 4.47 (H-6b); Glc B: 5.12 (H-1), 3.55 (H-2), 3.37 (H-3), 3.31 (H-4), 3.43 (H-5), 3.60 (H-6a), 3.83 (H-6b); Glc C: 4.86 (H-1), 3.18 (H-2), 3.31 (H-3), 3.23 (H-4), 3.31 (H-5), 4.10 (H-6a, -6b); ^{13}C NMR (125.65 MHz) (CF_3CO_2D-DMSO-d_6, 1:9): δ pelargonidin: 165.3 (C-2), 143.9 (C-3), 134.9 (C-4), 155.1 (C-5), 105.0 (C-6), 168.1 (C-7), 96.3 (C-8), 162.6 (C-9), 111.6 (C-10), 118.9 (C-1'), 134.9 (C-2'), 116.9 (C-3'), 155.1 (C-4'), 116.9 (C-5'), 134.9 (C-6'); caffeic acid: 128.5 (C-1), 116.2 (C-

2), 146.7 (C-3), 149.4 (C-4), 116.2 (C-5), 128.5 (C-6), 144.9 (C-α), 116.2 (C-β), 166.4 (C=O); caffeic acid: 125.7 (C-1), 115.2 (C-2), 145.5 (C-3), 148.3 (C-4), 115.7 (C-5), 125.7 (C-6), 145.4 (C-α), 113.9 (C-β), 166.1 (C=O); caffeic acid: 125.4 (C-1), 116.2 (C-2), 145.5 (C-3), 147.2 (C-4), 115.7 (C-5), 125.4 (C-6), 145.4 (C-α), 114.9 (C-β), 166.1 (C=O); Glc A: 100.0 (C-1), 80.7 (C-2), 75.6 (C-3), 73.3 (C-4), 74.0 (C-5), 63.1 (C-6); Glc B: 102.0 (C-1), 77.5 (C-2), 73.2 (C-3), 72.3 (C-5), 60.9 (C-6); Glc C: 104.1 (C-1), 74.2 (C-2), 69.5 (C-3), 70.0 (C-4), 69.7 (C-5), 62.8 (C-6); Glc D: 101.4 (C-1), 75.9 (C-2), 75.0 (C-5), 63.1 (C-6); Glc E: 102.0 (C-1), 76.2 (C-2), 60.9 (C-6).

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