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FLAVONOIDS ISOLATED FROM *DIPTEROCARPUS OBTUSIFOLIUS*

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Abstract

Five flavonoids including tiliroside (**1**), kaempferol 3-*O*-(2′′,6′′-di-*O*-(*E*)-*p*-coumaroyl-*β*-D-glucopyranoside) (**2**), myricetin (**3**), myricitrin (**4**), and myricetin 3-*O*-*α*-arabinofuranoside (**5**) have been isolated and identified from the leaves of *Dipterocarpus obtusifolius.* Their chemical structures were elucidated by spectroscopic methods including NMR and MS, and by comparison with the literature data. All these compounds were isolated from this plant for the first time.

Keywords. *Dipterocarpus obtusifolius*, Dipterocarpaceae, flavonoid.

1. INTRODUCTION

Dipterocarpus obtusifolius (Dipterocarpaceae) is a tree with the usual height of 20–25 m, which grows in clear forests in low regions up to 1500 m altitude, and widely distributed in countries such as Vietnam, Myanma, Cambodia, Laos, Thailand, and Malaysia. Traditionally, the oil of this plant is used in folk medicine as treatment of gonorrhea, pimples and skin diseases [1]. Previous phytochemical investigations on this species revealed that diterpenes, sesquiterpenes and triterpenes represented in the extract of the stems [2]. Chemical studies of the leaves of this plant have never been conducted previously. In this paper, we report the isolation and structural elucidation of five known flavonoids **1**–**5** from the *D. obtusifolius* leaves.

2. EXPERIMENTAL

2.1. General experimental procedures

The ESI-MS was measured on Agilent 1260 series single quadrupole LC/MS systems. NMR spectra were recorded on a Bruker AM500 FT-NMR spectrometer (Bruker, Billerica, MA, U.S.A.) using TMS as an internal Standard. Column chromatography (CC) was performed using a silica gel (Kieselgel 60, 70–230 mesh and 230–400 mesh, Merck, Darmstadt, Germany) or YMC RP-18 resins (30 - 50 μm, Fuji Silysia Chemical Ltd, Aichi, Japan). Thin layer chromatography (TLC) used pre-coated silica gel 60 F_{254} (1.05554.0001, Merck, Darmstadt, Germany) and RP-18 F_{254S} plates (1.15685.0001, Merck, Darmstadt, Germany) and compounds were visualized by spraying with aqueous 10 % H_2SO_4 and heating for 3–5 minutes.

2.2. Plant material

The samples of the plant *Dipterocarpus obtusifolius* Teijsm. ex Miq. were collected in May 2013 at Bao Lam, Lam Dong and identified by Dr. Nong Van Duy. A voucher specimen (No. TN3/242) was deposited at the Tay Nguyen Institute of Scientific Research, VAST.

2.3. Extraction and isolation

The air dried and powdered leaves of *D. obtusifolius* (4.5 kg) were extracted with methanol at 40^o C three times. Methanolic extracts were combined and evaporated under vacuum. This extract (500 g) was suspended in water and partitioned in turn with *n*-hexane, CH_2Cl_2, and EtOAc to provide the corresponding extracts: *n*-hexane (H, 37 g), CH_2Cl_2 (C, 26 g), EtOAc (E, 95 g) and a water layer.

Extract E was crudely separated by silica gel CC using a gradient concentration of MeOH in CH_2Cl_2 (0–100 %) to obtain nine fractions (E1–E9).

Fraction E6 (25 g) was subjected to a silica gel

CC and eluted with CH_2Cl_2/MeOH (from 20:1 to 0:1 v/v) to afford six subfractions E6A-E6F. Subfraction E6D (5 g) was subjected to column chromatography on a silica gel column and eluting with CH_2Cl_2/MeOH (10:1, v/v) to afford seven subfractions E6D1 – E6D7. Subfraction E6D2 was further purified by silica gel CC and eluted with *n*-hexane/EtOAc/MeOH (1:2:0.1, v/v/v) to obtain compound **2** (20 mg). Compound **3** (7.0 mg), compound **1** (200 mg), compound **5** (20 mg) were successively purified by recrystallization from subfraction E6D4, E6D5 and E6D6. Subfraction E6F was further purified by silica gel CC eluting with CH_2Cl_2/MeOH/H_2O (5:1:0.1) to give compound **4** (10 mg).

Tiliroside (**1**): Yellow needles; ^{1}H-NMR (500 MHz, CD_3OD) and ^{13}C-NMR (125 MHz, CD_3OD) see table 1; Positive ESI-MS *m/z* 595$[M+H]^+$.

Kaempferol 3-*O*-(2′′,6′′-di-*O*-(*E*)-*p*-coumaroyl-*β*-D-glucopyranoside) (**2**): Yellow amorphous powder; ^{1}H-NMR (500 MHz, CD_3OD) and ^{13}C-NMR (125 MHz, CD_3OD) see table 2. Positive ESI-MS *m/z* 741 $[M+H]^+$.

Myricetin (**3**): Yellow needles; ^{1}H-NMR (500 MHz, CD_3OD) and ^{13}C-NMR (125 MHz, CD_3OD) see table 3. Positive ESI-MS *m/z* 341 $[M+Na]^+$.

Myricitrin (**4**): Yellow needles; ^{1}H-NMR (500 MHz, CD_3OD) and ^{13}C-NMR (125 MHz, CD_3OD) see table 4. Positive ESI-MS *m/z* 487 $[M+Na]^+$.

Myricetin 3-*O*-*α*-arabinofuranoside (**5**): Light yellow amorphous powder, mp 241^{0}C; ^{1}H-NMR (500 MHz, CD_3OD) and ^{13}C-NMR (125 MHz, CD_3OD) see table 4. Positive ESI-MS *m/z* 451 $[M+H]^+$.

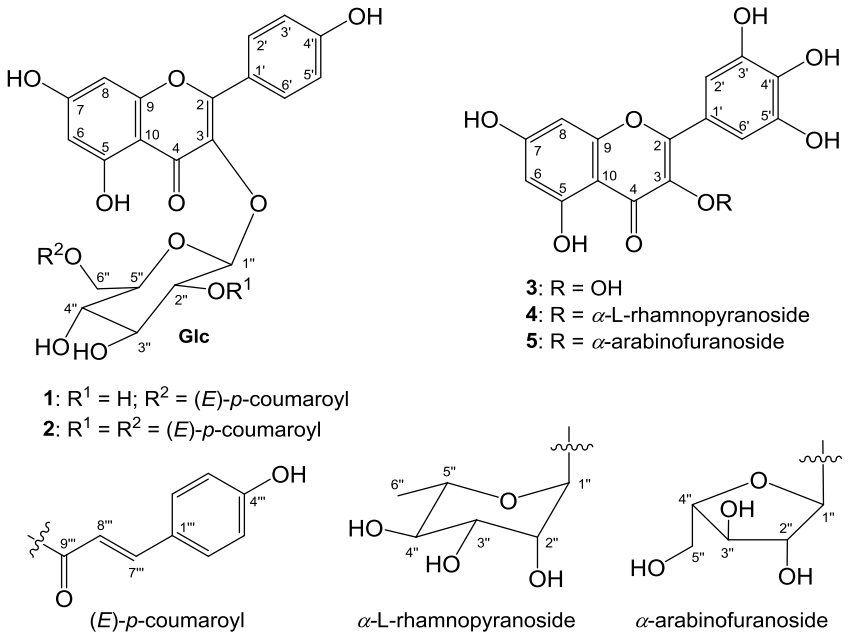

Figure 1: Structures of compounds **1**–**5**

3. RESULTS AND DISCUSSION

Compound **1** was obtained as yellow needles. A molecular formula of $C_{30}H_{26}O_{13}$ was determined for compound **1** on the basis of the observation of a molecular ion peak $[M + H]^+$ at *m/z* 595 in ESI-MS. The ^{1}H and ^{13}C NMR, and DEPT spectra of **1** suggested the presence of kaempferol glucoside derivative and a (*E*)-*p*-coumaroyl moiety. The ^{13}C-NMR spectrum exhibited 30 signals, including 5 sp^3 methine, 1 sp^3 methylene, 12 sp^2 methine, 10 sp^2 quaternary signals and 2 carbonyl carbons. In the ^{1}H-NMR spectrum, the *meta* coupled doublets at δ 6.15 (1H, d, *J* = 2.0 Hz) and 6.32 (1H, d, *J* = 2.0 Hz) were assigned to H-6 and H-8 of the A ring. Four aromatic proton signals at δ 6.83 (2H, d, *J* = 8.0 Hz, H-3', H-5'), 8.01 (2H, d, *J* = 8.0 Hz, H-2', H-6'), 6.80 (2H, d, *J* = 8.0 Hz, H-3''', H-5'''), and 7.32 (2H, d, *J* = 8.0 Hz, H-2''', H-6'''), characterize two AA′BB′ spin systems of the flavonoid B ring and the coumaroyl group. In addition, the signals at δ 6.18 (1H, d, *J* = 16.0 Hz) and 7.42 (1H, d, *J* = 16.0 Hz) of two *trans* olefinic protons were attributed to H-8''' and H-7''' of (*E*)-*p*-coumaroyl moiety, on basis of their long-range correlations with C-1''' (δ 127.09), C-2''', C-6''' (δ 131.15) and C-9''' (δ 168.78). The ^{1}H-NMR spectrum also supported the presence of one *β*-D-glucose moiety with an anomeric proton signal

at δ 5.26 (1H, d, *J* = 7.5 Hz, H-1"). The HMBC correlations between H-1" and C-3 (δ 135.20), and between H-6" (δ 4.21/4.32) and C-9'" (δ 168.78) indicated that the sugar unit attached at the C-3 position and the (*E*)-*p*-coumaroyl group linked to C-6". Based on these evidences and comparison with the reported values in literature [3], the structure of **1** was assigned as tiliroside.

Table 1: The ^{1}H (500 MHz, CD_3OD) and ^{13}C-NMR (125 MHz, CD_3OD) data of compound **1**

C	$^{a}\delta_C$	δ_C	δ_H ^{a} (mult., *J* = Hz)	C	$^{a}\delta_C$	δ_C	δ_H (mult., *J* = Hz)
2	156.3	159.34	-	1"	101.0	103.97	5.26 (1H, d, 7.5)
3	133.1	135.20	-	2"	74.2	75.72	3.50 (1H)*
4	177.4	179.42	-	3"	76.2	78.01	3.47 (1H)*
5	156.3	162.95	-	4"	69.9	71.72	3.33 (1H)*
6	98.7	99.98	6.15 (1H, d, 2.0)	5"	74.2	75.80	3.51 (1H)*
7	164.1	165.92	-	6"	63.0	64.31	4.21 (1H, dd, 6.5, 12.0) 4.32 (1H, dd, 2.5, 12.0)
8	93.6	94.82	6.32 (1H, d, 2.0)	1'"	124.9	127.09	-
9	161.1	158.40	-	2'", 6'"	130.1	131.15	7.32 (2H, d, 8.0)
10	103.9	105.60	-	3'", 5'"	115.7	116.78	6.80 (2H, d, 8.0)
1'	120.8	122.73	-	4'"	159.7	161.16	-
2', 6'	130.8	132.19	8.01 (2H, d, 8.0)	7'"	144.5	146.53	7.42 (1H, d, 16.0)
3', 5'	115.0	116.03	6.83 (2H, d, 8.0)	8'"	113.6	114.75	6.18 (1H, d, 16.0)
4'	159.9	161.50	-	9'"	166.1	168.78	-

$^{a}\delta_C$ of tiliroside recorded in DMSO-d_6 [3], *overlapped signals.

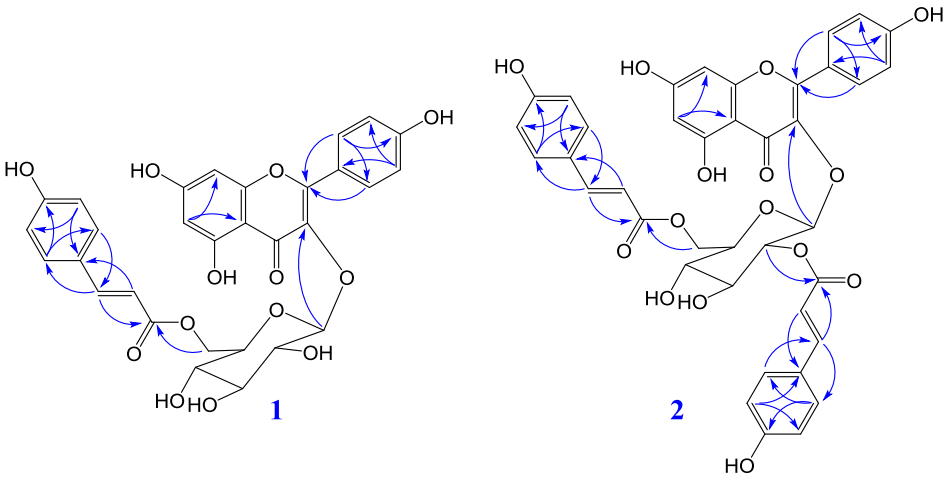

Figure 2: Key HMBC correlations of compounds **1** and **2**

Compound **2** was isolated as yellow amorphous powder. Its molecular formula was established as $C_{39}H_{32}O_{15}$ on the basis of an ion peak $[M + H]^+$ at *m/z* 741 in ESI-MS. The ^{1}H and ^{13}C-NMR spectral data of **2** were very similar to those of **1** (see table 2), except for the appearance of an additional AA′BB′ spin systems at δ 6.82 (2H, d, *J* = 8.7 Hz, H-3'", H-5'"), 7.32 (2H, d, *J* = 8.7 Hz, H-2'", H-6'"), a pair of olefinic doublets at δ 6.09 (1H, d, *J* = 16.0 Hz, H-8'") and 7.42 (1H, d, *J* = 16.0 Hz, H-7'") and of nine additional carbon signals at δ 127.08 (C-1'"), 131.19 (C-2'", C-6'"), 116.82 (C-3'", C-5'"), 161.15 (C-4'"), 146.56 (C-7'"), 114.67 (C-8'"), 168.74 (C-9'"), which indicate that **2** contained two (*E*)-*p*-coumaroyl moieties. The downfield-shift of the H-2" signals at δ 5.10 (1H, dd, *J* = 8.0, 9.0 Hz) suggested that the (*E*)-*p*-coumaroyl unit was attached at the C-2" position of the glucopyranosyl moiety. This was also confirmed by the cross peak between H-2" (δ 5.10) and C-9'" (δ 168.74), and between H-7'" (δ 7.42) and C-9'" (δ 168.74), C-8'" (δ 114.67), C-2'", C-6'" (δ 131.19) in the HMBC spectrum. Thus, **2** was identified to be kaempferol 3-*O*-(2",6"-di-*O*-(*E*)-*p*-coumaroyl-*β*-D-glucopyranoside) by comparison with the reported chemical data [4].

Table 2: The ^{1}H (500 MHz, CD_3OD) and ^{13}C-NMR (125 MHz, CD_3OD) data of compound **2**

C	$^a\delta_C$	δ_C	δ_H (mult., *J* = Hz)	C	$^a\delta_C$	δ_C	δ_H (mult., *J* = Hz)
2	156.36	158.96	-	5"	73.90	75.91	3.60 (1H, m)
3	132.57	134.45	-	6"	62.84	64.13	4.23 (1H, dd, 6.5, 12.0) 4.38 (1H, dd, 2.0, 12.0)
4	177.12	179.13	-	1'''	125.00	127.08	-
5	161.18	162.98	-	2''', 6'''	130.30	131.19	7.32 (1H, d, 8.7)
6	98.36	99.88	6.08 (1H, d, 2.2)	3''', 5'''	115.88	116.82	6.82 (1H, d, 8.7)
7	164.30	165.64	-	4'''	160.13	161.15	-
8	93.75	94.69	6.28 (1H, d, 2.2)	7'''	144.82	146.56	7.42 (1H, d, 16.0)
9	156.48	158.29	-	8'''	113.64	114.67	6.09 (1H, d, 16.0)
10	103.88	105.73	-	9'''	165.97	168.74	-
1'	120.69	122.87	-	1''''	125.20	127.33	-
2', 6'	130.90	132.16	7.97 (1H, d, 8.7)	2'''', 6''''	130.30	131.24	7.50 (1H, d, 8.7)
3', 5'	115.18	116.13	6.89 (1H, d, 8.7)	3'''', 5''''	115.88	116.79	6.83 (1H, d, 8.7)
4'	159.90	161.36	-	4''''	159.94	161.23	-
1"	98.84	100.38	5.67 (1H, d, 8.0)	7''''	145.24	147.05	7.74 (1H, d, 16.0)
2"	73.90	75.63	5.10 (1H, dd, 8.0, 9.0)	8''''	114.33	115.27	6.45 (1H, d, 16.0)
3"	74.38	76.12	3.71 (1H, t, 9.0)	9''''	166.24	168.55	-
4"	70.24	71.98	3.45 (1H, t, 9.0)				

$^a\delta_C$ of kaempferol 3-*O*-(2′′,6′′-di-*O*-(*E*)-*p*-coumaroyl-*β*-D-glucopyranoside) recorded in DMSO-d_6 [4].

Compound **3** was obtained as yellow needles. The ESI mass spectrum of **3** exhibited an ion peak $[M+Na]^+$ at *m/z* 341, which is in agreement with the molecular formula $C_{15}H_{10}O_8$. The ^{13}C-NMR and DEPT spectra showed 15 signals comprising one carbonyl carbon, 4 sp^2 methine, 10 sp^2 quaternary signals. The ^{1}H-NMR spectrum exhibited a characteristic *meta*-coupled proton signal at δ 6.19 (1H, d, *J* = 2.0 Hz) and 6.38 (1H, d, *J* = 2.0 Hz) corresponding to H-6 and H-8 of flavonoid A ring. The other AX coupling system at δ 7.36 (2H, br s) was assigned to H-2' and H-6' of B ring. By comparing the NMR spectral data with those reported in literature, the structure of **3** was determined as myricetin [5].

Table 3: The ^{1}H (500 MHz, CD_3OD) and ^{13}C-NMR (125 MHz, CD_3OD) data of compound **3**

C	$^a\delta_C$	δ_C	δ_H (mult., *J* = Hz)	C	$^a\delta_C$	δ_C	δ_H (mult., *J* = Hz)
2	146.8	147.91	-	9	156.1	158.09	-
3	135.8	137.29	-	10	103.0	104.44	-
4	175.7	177.18	-	1'	120.8	123.05	-
5	160.7	162.35	-	2', 6'	107.2	108.53	7.36 (2H, brs)
6	98.1	99.21	6.19 (1H, d, 2.0)	3', 5'	145.7	146.65	-
7	163.8	165.46	-	4'	135.8	136.88	-
8	93.2	94.37	6.38 (1H, d, 2.0)				

$^a\delta_C$ of myricetin recorded in DMSO-d_6 [5].

Compound **4** was obtained as yellow needles. Its molecular formula was established as $C_{21}H_{20}O_{12}$ on the basis of an ion peak $[M+Na]^+$ at *m/z* 487 in ESI-MS. The spectroscopic data of **4** were similar to **3** (see table 4), except for the appearance of an α-L-rhamnopyranosyl moiety. So, the ^{1}H-NMR spectrum of **4** showed the presence of an anomeric proton signal at δ 5.33 (1H, br s), a methyl signal at δ 0.99 (3H, d, *J* = 6.5 Hz) and of six additional carbon signals at δ 103.60 (C-1"), 71.89 (C-2"), 72.02 (C-3"), 73.37 (C-4"), 72.13 (C-5"), and 17.66 (C-6"). The position of the sugar unit in compound **4** was confirmed by the HMBC correlation between H-1" (δ 5.33) and C-3 (δ 136.19). These data and other NMR data thus allowed us to identify compound **4** as myricitrin [6].

Compound **5** was isolated as light yellow amorphous powder. The ^{1}H and ^{13}C-NMR spectra of

5 were very similar to those of **3**, except for the appearance of an additional *α*-arabinofuranose moiety that was attached at C-3. Accordingly, the structure of **5** was established as myricetin 3-*O*-*α*-arabinofuranoside [7].

Table 4: The ^{1}H (500 MHz, CD_3OD) and ^{13}C-NMR (125 MHz, CD_3OD) data of compounds **4** and **5**

C	$^{a}\delta_C$	4		$^{b}\delta_C$	5	
		δ_C	δ_H (mult., *J* = Hz)		δ_C	δ_H (mult., *J* = Hz)
2	157.47	159.15		-	159.41	
3	134.20	136.19		-	134.90	
4	177.77	179.51		-	180.02	
5	161.30	163.05		-	163.04	
6	98.60	100.29	6.17 (1H, d, 2.0)	-	99.88	6.22 (1H, d, 2.0)
7	164.20	167.36		-	166.06	
8	93.50	95.03	6.32 (1H, d, 2.0)	-	94.75	6.40 (1H, d, 2.0)
9	156.40	158.61		-	158.55	
10	104.01	105.46		-	105.57	
1'	119.59	121.92		-	122.01	
2', 6'	107.88	109.55	6.96 (1H, br s)	-	109.45	7.15 (1H, s)
3', 5'	145.76	146.87		-	146.84	
4'	136.44	137.99		-	137.96	
1"	101.93	103.60	5.33 (1H, br s)	109.5	109.42	5.49 (1H, br s)
2"	70.00	71.89	4.24 (1H, br s)	83.3	83.26	4.36 (1H, d, 2.0)
3"	70.37	72.02	3.81 (1H, br d, 7.5)	78.6	78.77	3.93 (1H, m)
4"	71.26	73.37	3.36*	87.9	88.04	3.95 (1H, m)
5"	70.55	72.13	3.54 (1H, m)	62.5	62.57	3.55 (2H, m)
6"	17.50	17.66	0.99 (3H, d, 6.5)			

$^{a}\delta_C$ of myricitrin recorded in DMSO-d_6 [6], $^{b}\delta_C$ of quercetin 3-*O*-*α*-arabinofuranoside recorded in CD_3OD [7], *overlapped signals.

4. CONCLUSION

From the MeOH extract of the leaves of *Dipterocarpus obtusifolius*, using column chromatography, five known compounds tiliroside (**1**), kaempferol 3-O-(2′′,6′′-di-O-(*E*)-*p*-coumaroyl-*β*-D-glucopyranoside) (**2**), myricetin (**3**), myricitrin (**4**), myricetin 3-*O*-*α*-arabinofuranoside (**5**) were isolated. Based on 1D-NMR and 2D-NMR as well as comparison with published data, their chemical structures were elucidated. This is the first report of these compounds from this species.

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REFERENCES

1. V. V. Chi. *Dictionary of Vietnamese medicinal plants 1*, Hanoi, Publishing House Medicine, 722 (2012).
2. P. Khiev, O. K. Kwon, H. H. Song, S. R. Oh, K. S. Ahn, H. K. Lee, Y. W. Chin. *Cytotoxic terpenes from the stems of Dipterocarpus obtusifolius collected in Cambodia*, Chem Pharm Bull (Tokyo), **60(8)**, 955-961 (2012).
3. M. Kuroyanagi, M. Fukuoka, K. Yoshihira, S. Natori, K. Yamasaki. *Confirmation of the structure of Tiliroside, an acylated kaempferol glycoside, by ^{13}C-Nuclear magnetic resonance*, Chemical & pharmaceutical bulletin, **26(11)**, 3594-3596 (1978).
4. N. Yamashita, H. Etoh, K. Sakata, A. Yagi, H. Ina, K. Ina. *An acylated kaempferol glucoside isolated from Quercus dentata as a repellent against the blue mussel Mytilus edulis*, Agricultural and Biological Chemistry, **53(5)**, 1383-1385 (1989).

5. Y. -S. C. Chien-Chang Shen, Li-Kang Hott. *Nuclear magnetic resonance studies of 5,7-dihydroxyflavonoids*, Phytochemistry, **34**, 843-845 (1993).
6. M. M. S. Mahmoud I. I., Moharram F. A., El-Gindi M. R., Hassan A. M. *Acylated flavonol glycosides from Eugenia jambolana leaves*, Phytochemistry, **58**, 1239-1244 (2001).
7. M. Kalegari, M. D. Miguel, J. d. F. G. Dias, A. L. L. Lordello, C. P. d. Lima, C. M. S. Miyazaki, S. M. W. Zanin, M. C. d. S. Verdam, O. G. Miguel. *Phytochemical constituents and preliminary toxicity evaluation of leaves from Rourea induta Planch. (Connaraceae)*, Brazilian Journal of Pharmaceutical Sciences, **47**, 635-642 (2011).

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