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ANTIOXIDANT ACTIVITY OF A NEW FLAVONE GLYCOSIDE FROM THE STEMS OF *GMELINA ARBOREA* ROXB.

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Keywords:

Gmelina arborea Roxb., Verbenaceae, Flavone glycoside, Luteolin, Apigenin, DPPH assay

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ABSTRACT: *Gmelina arborea* Roxb. belongs to the Verbenaceae family, commonly known as Gambhar. It is distributed throughout India, Ceylon, Malayan and Philippine Islands. Its roots have anthelmintic properties. Its flowers are useful in the treatment of leprosy and blood diseases. Its fruit has diuretic properties and aphrodisiac. It is also used in anemia leprosy and vaginal discharges. Leaves have shown analgesic, antimicrobial, and anti-diabetic activities. A new flavone glycosides 5, 7.4′-trihydroxy-8, 3′-dimethoxyflavone- 4′-O-β-D-glucopyranosyl- 7-O-β-D-xylopyranosyl (1→2)-α-L-rhamnopyranoside(A) along with two known compounds Luteolin (B) and Apigenin (C) were isolated from ethanolic extract of the stem parts of the plant. The characterization and structural elucidation of these compounds were carried out by various color reactions, chemical degradation, and spectral analysis. Antioxidant activity of compound A was evaluated by 1, 1-diphenyl-2-pycrylhydrazyl (DPPH) assay system which has shown a significant radical scavenging activity.

INTRODUCTION: *Gmelina arborea* Roxb. (Hort. Beng. 1814) belongs to Verbenaceae family and commonly known as Gambhar [1, 2]. It is a moderate size unarmed deciduous tree, reaching 18m high, bark greyish-yellow, leaves 10-20 by 7.5-15 cm broadly ovate and petioles 5-7.5 cm long, cylindric and glandular at the top. It is distributed throughout India, Ceylon, Malayan, and Philippine Islands. Its roots have anthelmintic properties. Its flowers are useful in the treatment of leprosy and blood diseases. Its fruit has diuretic properties and aphrodisiac. It is also used in anemia leprosy and vaginal discharges.

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Leaves have shown analgesic, antimicrobial and anti-diabetic activities [1]. Various bioactive constituents have been reported from this plant by earlier workers [3, 4, 5, 6]. In this paper we deal with the isolation and antioxidant activity of a new flavone glycoside (A) 5, 7.4′-trihydroxy-8, 3′-dimethoxyflavone- 4′-O-β-D-glucopyranosyl-7-O-β-D-xylopyranosyl (1→2)-α-L-rhamnopyranoside from ethanolic extract of stem parts of the plant along with two known compound B and C.

MATERIALS AND METHODS:

General Experimental Procedure: All the melting points were determined on a thermo-electrically melting point apparatus and are uncorrected. The IR spectra were recorded at Shimadzu FT-IR 8400S in KBr disc. UV spectra were determined on Shimadzu-120 double beam spectrometer in MeOH. ^{1}H-NMR spectra were recorded on Bruker DRX 300 MHz spectrometer in

$CDCl_3$ using TMS as an internal standard. ^{13}C-NMR spectra were recorded on Bruker DRX 75 MHz spectrometer using $CDCl_3$. The chemical shift values are reported in ppm (δ) units and coupling constants (J) in Hz. The FAB mass spectra were recorded on Jeol-SX (102) Mass spectrometer.

Plant Material: The stems of the plant were collected locally around Sagar region and identified by a taxonomist, Department of Botany, Dr. H. S. Gour Vishwavidyalaya, Sagar, Madhya Pradesh, India. A voucher specimen (Bot/H/05/12/05) has been deposited in Natural Products Laboratory, Department of Chemistry of this university.

Extraction and Isolation: Air-dried powdered stem parts of the plant (5 kg) were extracted with ethanol in Soxhlet apparatus for 5-7 days. The ethanolic extract obtained was concentrated under reduced pressure to give brown viscous mass, which was further partitioned with solvents of increasing polarity, pet. ether, $CHCl_3$, EtOAc, Me_2CO, and MeOH. The methanol soluble fraction was concentrated under reduced pressure by a rotatory evaporator to obtain the brown viscous mass of 3.95 gm. On TLC examination over silica Gel-G using nBAW (4:1:5) as solvent and I_2 vapors as visualizing agent showed three spots indicating it to be a mixture of three compounds A, B and C which were further purified by column chromatography over a SiO_2-gel column using $CHCl_3$: MeOH in various proportions (8:2, 7:3, 9:1). The solvents of the eluents obtained were removed and crystallized from the ether, which yielded compound A (1.78 gm), compound B (0.52 gm) and compound C (0.71 gm) respectively.

RESULTS AND DISCUSSION: Compound A has m.p. 272-273°C, m.f. $C_{34}H_{42}O_{20}$, $[M^+]$ 770 (FABMS). It gave Molisch and Shinoda [7, 8] tests confirming its flavonoids glycosidic nature. IR (KBr) ν_{max} cm^{-1} 3450 (-OH), 2988, 2917 (-CH stretching), 2872 (-OMe), 1649 (-C=O), 1623 (aromatic ring system) and 1525, 1478, 1132, 1015 and 830 cm^{-1}. In ^{1}HNMR spectrum of compound A a singlet at δ 6.58 (1H, s) was assigned for H-6 in ring A, and a singlet at δ 6.71 (1H, s) for H-3 in ring C. Two doublets at δ 7.78 (1H, J 2 Hz) and δ 6.61 (1H, J 8.6 Hz) were assigned for H-2′ and H-5′ in ring B. A double doublet at δ 7.64 (1H, dd, J 8.8, 2.2 Hz) was assigned for H-6′ in ring B. A singlet was assigned for –OH group at C-5 position at δ 12.24. Two singlets at δ 3.78 and 3.73 show the presence of $–OCH_3$ group at C-8 and C-3′. The anomeric proton signals at δ 6.02 (1H, s, J 1.6Hz), δ 5.03 (1H, d, J 7.4 Hz) and δ 5.88 (1H, d, J 7.2 Hz) were assigned for H-1″ of L-rhamnose, H-1‴ of D-xylose and H-1⁗ of D-glucose respectively.

A coupling constant at J 1.6 Hz of H-1″ confirmed the α-configuration for L-rhamnose and coupling constant at 7.4 Hz and 7.2 Hz confirmed the β-configuration for D-xylose and D-glucose respectively [9, 10]. The characteristic ion peaks in the mass spectrum of compound A observed at m/z 770 $[M^+]$, 608 [M^+-D-Glucose], 476 [M^+-D-Xylose] and 330 [M^+-L-rhamnose and aglycone] were obtained by subsequent losses from the molecular ion of each molecule of D-glucose, D-xylose and L-rhamnose indicating D-glucose at C-4′ position, D-xylose and L-rhamnose were linked to aglycone at C-7 position. Acid hydrolysis of compound A with 10% ethanolic H_2SO_4 gave aglycone A-1, m.p. 229-230 °C, m.f. $C_{17}H_{14}O_7$ $[M^+]$ 330 (FABMS) which was identified as 5,7, 4′-trihydroxy-8,3′-dimethoxy flavone.

The aqueous hydrolysate obtained on acid hydrolysis of compound A was neutralized with $BaCO_3$ and $BaSO_4$ was filtered off. The filtrate obtained was concentrated and subjected to paper chromatography, examination showed the presence of L-rhamnose (R_f 0.37), D-xylose (R_f 0.28) and D-glucose (R_f 0.16). Periodate oxidation [11, 12] of compound A confirmed that all sugars were present in pyranose form. Permethylation [13, 14] followed by acid hydrolysis, yielded methylated aglycone and methylated sugars confirmed the presence of methoxy group in compound A. The methylated aglycone was identified as 7, 4′-dihydroxy-5, 8, 3′-trimethoxy flavone which confirmed that glycosidation was involved at C-7-OH and C-3′-OH positions of aglycone. The methylated sugars were identified as 2,3,4,6-tetramethoxy glucose (R_G 1.0), 3,4-dimethoxy rhamnose (R_G 0.84) and 2,3,4-trimethoxy xylose (R_G 0.94).

Therefore it was concluded that C-1⁗-OH of D-glucose was attached with OH group at C-4′ position of aglycone, C-1″-OH of L-rhamnose was attached with –OH group at C-7 of aglycone, C-2″-OH of L-rhamnose was linked with C-1‴-OH of D-

xylose. Thus interglycosidic linkage (1→2) was found between L-rhamnose and D-xylose. Enzymatic hydrolysis[15] of compound A by almond emulsion shows β-linkage of D-xylose with L-rhamnose and that of D-glucose with aglycone and further hydrolysis of proaglycone by Takadiastase enzyme liberated L-rhamnose showing α-linkage with aglycone. On the basis of above evidence compound A was identified as 5, 7.4′-trihydroxy-8, 3′-dimethoxyflavone- 4′-O-β-D-glucopyranosyl-7-O-β-D-xylopyranosyl (1→2)-α-L-rhamnopyranoside. Compound B and C have been reported as Luteolin and Apigenin and compound A has been evaluated for its antioxidant activity (See in the experimental section).

Study of Compound A: It was analyzed for m.f. $C_{34}H_{42}O_{20}$, m.p. 272-273 °C, [M^+] 770 found (%) C 53.12, H 5.69, O 41.61 and calcd. form.f. $C_{34}H_{42}O_{20}$ found (%) C 53.17, H 5.71, O 41.66. UV (MeOH) λ_{max} nm 242, 276 and 350. IR (KBr) ν_{max} (cm^{-1})- 3450, 2988, 2917, 2872, 1649, 1623, 1525, 1478, 1132, 1015 and 830. ^{1}HNMR (300 MHz, $CDCl_3$); 12.24 (1H, s, 5-OH), 6.58 (1H, s, H-6), 6.71 (1H, s, H-3), 7.78 (1H, d, J 2 Hz, H-2′), 6.61 (1H , d, J 8.6 Hz , H-5′), 7.64 (1H, dd, J 8.8, 2.2 Hz, H-6′), 3.78 (OMe-C-8), 3.73 (OMe-C-3′), 6.02 (1H, br, s, H-1″), 4.12 (1H, d, J 3.6 Hz, H-2″), 3.68 (1H, dd, H-3″), 3.40 (1H, dd, H-4″), 3.85 (1H, m, H-5″), 1.12 (3H, d, J 6.2 Hz, CH_3), 5.03 (1H, d, J 7.4 Hz, H-1‴), 3.75 (1H, dd, H-2‴), 3.49 (1H, dd, H-3‴), 3.41(1H, m, H-4‴), 3.21 (1H, dd, J 12.2, 9.9 Hz, H-5_a‴), 3.63 (1H, m, H-5_b‴), 5.88 (1H, d, J 7.2 Hz, H-1⁗), 3.65 (1H, dd, H-2⁗), 3.48 (1H, dd,H-3⁗), 3.43 (1H, dd, H-4⁗), 3.26 (1H, m, H-5⁗), 3.46 (1H, dd, J 12.0 5.2 Hz, H-6_a⁗), 3.60 (1H, dd, J 12.0, 2.2 Hz, H-6_b⁗). ^{13}C-NMR (75 MHz, $CDCl_3$) 168.8 (C-2), 104.9 (C-3), 187.0 (C-4) 152.1 (C-5), 102.3 (C-6), 152.3 (C-7) 129.3 (C-8), 145.5 (C-9), 105.0 (C-10), 127.5 (C-1′), 112.4 (C-2′), 147.6 (C-3′), 143.6 (C-4′), 115.1 (C-5′), 119.1 (C-6′), 56.6 (OMe-8), 56.3 (OMe-3′), 100.3 (C-1″), 87.3 (C-2″), 70.8 (C-3″), 74.6 (C-4″), 72.1 (C-5″), 18.5 (C-6″), 107.3 (C-1‴) 73.2 (C-2‴), 76.4 (C-3‴), 71.2 (C-4‴), 67.7 (C-5‴), 101.5 (C-1⁗), 74.1 (C-2⁗), 70.2 (C-3⁗), 69.1 (C-4⁗), 73.4 (C-5⁗), 60.5(C-6⁗).

Acid Hydrolysis of Compound A: 80 mg of compound A was dissolved in ethanol (15 ml) and refluxed with 10% H_2SO_4 for 7 h. The contents were concentrated, cooled and the residue was further extracted with diethyl ether. After washing the ether layer with water, the residue was chromatographed over silica gel using $CHCl_3$: MeOH as a solvent to give aglycone. This was identified as 5, 7, 4'-trihydroxy-8, 3'-dimethoxy flavone.

Permethylation of Compound A: Compound A (40 mg) was dissolved in DMF (20 ml) and refluxed for 48 hr with MeI (5 ml) and Ag_2O (40 mg), then filtered and washed with DMF. The filtrate was dried and hydrolyzed with 10% H_2SO_4 to obtain methylated aglycone, 7, 4'-dihydroxy-5, 8,3'-trimethoxy flavone. The aqueous hydrolysate so obtained after removal of aglycone was neutralized with $BaCO_3$ and $BaSO_4$ was filtered off. The filtrate was dried in vacuum and subjected to paper chromatography using nBAW (4:1:5) as a solvent and aniline hydrogen phthalate was used for detection. The methylated sugars were identified as 2,3,4,6-tetramethoxy glucose (R_G 1.0), 3, 4-dimethoxy rhamnose (R_G 0.84) and 2,3,4-trimethoxy xylose (R_G 0.94).

Enzymatic Hydrolysis of Compound A: Compound A (40 mg) was dissolved in MeOH (20 ml) and hydrolyzed with an equal volume of Takadiastase enzyme, which liberated L-rhamnose (R_f 0.37), showing α-linkage with aglycone. Further hydrolysis of glycoside by almond emulsion, shows β-linkage of D-glucose (R_f 0.18) with aglycone and that of xylose (R_f 0.28) with L-rhamnose.

Study of Compound A-1: It was analysed for m.f. $C_{17}H_{14}O_7$, m.p. 229-230 °C, [M^+] 330 found (%) C 62.02, H 4.28, O 34.01, Calcd. form.f. $C_{17}H_{14}O_7$ found (%) C 62.06, H 4.33, O 34.04 . UV (MeOH) λ_{max} (nm) 250, 287, 375. IR (KBr) ν_{max} (cm^{-1})- 3450, 2988, 2917, 2872, 1649, 1623, 1525, 1478. ^{1}HNMR (300 MHz, $CDCl_3$); 12.24 (1H, s, 5-OH), 6.58 (1H, s, H-6), 6.71 (1H, s, H-3), 7.78 (1H, d, J 2Hz, H-2′), 6.61 (1H, d, J 8.6 Hz, H-5′), 7.64 (1H, dd, J 8.8, 2.2 Hz, H-6′), 3.78 (OMe-C-8), 3.73 (OMe-C-3′). ^{13}C-NMR (75 MHz, $CDCl_3$) 168.8 (C-2), 104.9 (C-3), 187.0 (C-4) 152.1 (C-5), 102.3 (C-6), 152.3 (C-7) 129.3 (C-8), 145.5 (C-9), 105.0 (C-10), 127.5 (C-1′) 112.4 (C-2′), 147.6 (C-3′), 143.6 (C-4′), 115.1 (C-5′), 119.1 (C-6′), 56.6 (OMe-8), 56.3 (OMe-3′).

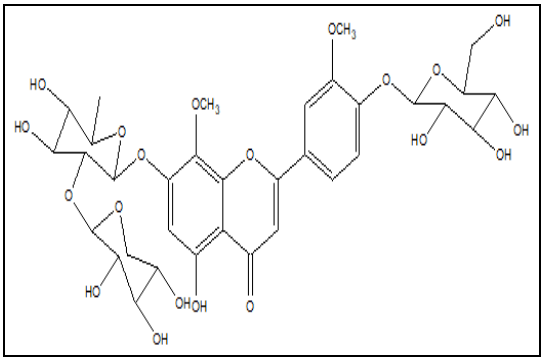

FIG. 1: COMPOUND A

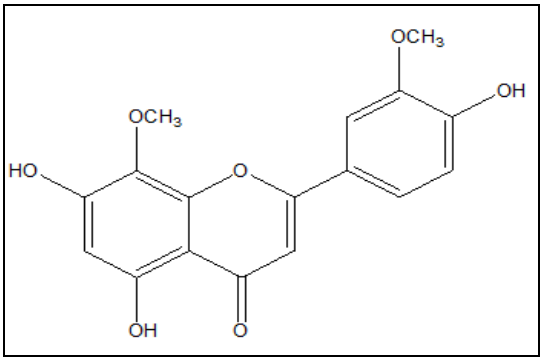

FIG. 2: COMPOUND A-1

Study of Compound B: It was analysed for m.f. $C_{15}H_{10}O_6$, m.p.-329-330 °C, found (%) C 62.78, H 3.84, O 33.40, Calcd. form.f. $C_{15}H_{10}O_6$ found (%) C 62.74, H 3.82, O 33.43. UV (MeOH) λ_{max} (nm) 253, 265, 348. IR (KBr) ν_{max} (cm^{-1})- 3394, 1638, 1600, 1560, 1517. ^{1}HNMR (300 MHz, $CDCl_3$); δ 12.94 (1H, s, 5-OH), 6.72 (1H, s, H-3), 6.48 (1H, d, J 2 Hz, H-6), 7.08 (1H, d, J 2 Hz, H-8), 7.76 (1H, br, s, H-2′), 6.87 (1H, d, J 8.3 Hz, H-5′), 7.42 (1H, dd, J 8.3, 2.2 Hz, H-6′). ^{13}C-NMR (75 MHz, $CDCl_3$); 163.40 (C-2), 102.90 (C-3), 181.81 (C-4), 161.80 (C-5), 101.09 (C-6), 162.48 (C-7), 95.50 (C-8), 158.32(C-9), 105.47 (C-10), 121.11 (C-1′), 114.12 (C-2′), 143.78 (C-3′),149.72 (C-4′), 116.70 (C-5′), 118.42 (C-6′). It was identified as Luteolin **Fig. 3** on comparison with reported literature values [16].

Study of Compound C: It was analysed for m.f. $C_{15}H_{10}O_5$, m.p.-346-347 °C, found (%) C 66.42, H 4.02, O 29.48, Calcd. form.f $C_{15}H_{10}O_5$ found (%) C 66.44, H 4.0, O 29.50. UV (MeOH) λ_{max} (nm) 266, 336. IR (KBr) ν_{max} (cm^{-1})- 3332, 1644, 1577, 1497, 1464. ^{1}HNMR (300 MHz, $CDCl_3$); δ 12.93 (1H, s, 5-OH), 7.94 (2H, d, J 8.6 Hz, H-2′, H-6′), 6.92 (2H, d, J 8.6 Hz, H-3′, H-5′), 6.82 (1H, s, H-3), 6.86 (1H, d, J 2 Hz, H-8), 6.44 (1H, d, J 2 Hz, H-6). ^{13}C-NMR (75 MHz, $CDCl_3$) ; 163.70 (C-2) , 101.82 (C-3), 182.75 (C-4), 162.42 (C-5), 100.12 (C-6), 164.32 (C-7), 94.88 (C-8), 157.34 (C-9), 102.68 (C-10), 122.15(C-1′), 130.42 (C-2′,C-6′), 115.9 (C-3′,C-5′) , 162.20 (C-4′). It was identified Apigenin **Fig. 4** on comparison with reported literature values [16].

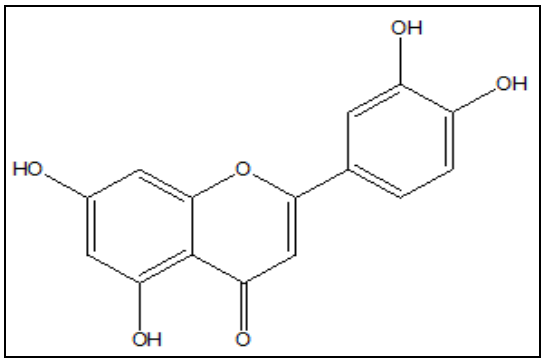

FIG. 3: COMPOUND B

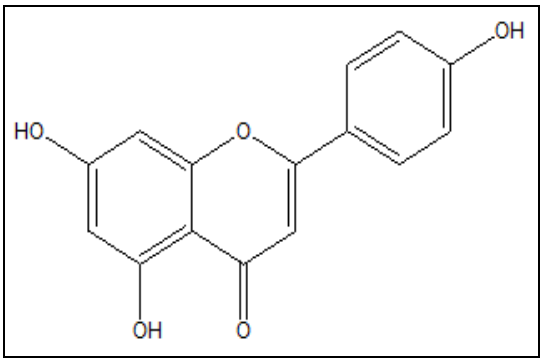

FIG. 4: COMPOUND C

Antioxidant Activity of Compound A: Free radical scavenging activity of compound A was evaluated by DPPH assay method [17]. 3 ml of DPPH solution (0.1 mM) was mixed with 2 ml of each solution (5-100 µg/ml) of the compound and allowed to incubate for 30 min in the darkroom. After the incubation of 30 min, the decrease in absorbance of DPPH solution was measured at 517 nm against the blank. 99% methanol served as blank. Control solution contains the DPPH solution and methanol. Ascorbic acid was taken as the standard. % inhibition of the DPPH free radical was calculated by the following equation:-

$$\% \text{ Inhibition} = (A_o - A_s / A_o) \times 100$$

Where A_o is the absorbance of the control and A_s is the absorbance of the sample.

Absorbance of control = 0.915.

TABLE 1: ANTIOXIDANT ACTIVITY OF COMPOUND A

Concentration µg/ml	Absorbance	% Inhibition
5	0.81	11.47
10	0.689	24.69
20	0.598	34.64
40	0.522	42.95
60	0.408	55.4
80	0.25	72.67
100	0.038	95.84

TABLE 2: ANTIOXIDANT ACTIVITY OF ASCORBIC ACID

Concentration µg/ml	Absorbance	% Inhibition
5	0.903	1.31
10	0.812	11.25
20	0.627	31.47
40	0.521	43.06
60	0.462	49.5
80	0.312	65.9
100	0.071	92.24

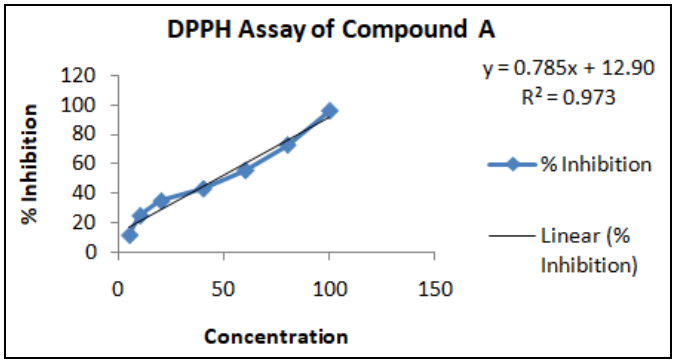

FIG. 5: ANTIOXIDANT ACTIVITY OF COMPOUND A

IC_{50} value of compound A is 47.24

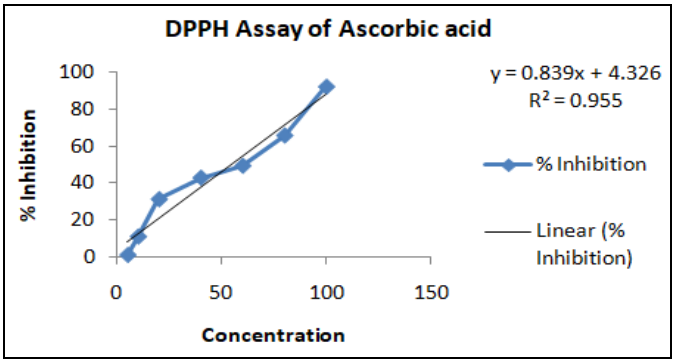

FIG. 6: ANTIOXIDANT ACTIVITY OF ASCORBIC ACID

IC_{50} value of ascorbic acid is 54.40

CONCLUSION: On the basis of above evidence the structure of compound (A) was established as 5,7.4'-trihydroxy-8, 3'-dimethoxyflavone-4'-O-β-D-glucopyranosyl- 7-O-β-D-xylopyranosyl(1→2)-α-L-rhamnopyranosidefrom the ethanolic extract of stems part of the plant. Compound (A) showed significant antioxidant activity and thus it may be used as a potent antioxidative agent.

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CONFLICTS OF STATEMENT: The authors hereby declare no conflict of interest in the publication of this paper.

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