Original Research Article

Chemical constituents from the stem bark of *Ficusthonningii* and their chemotaxonomic significance

Abstract

Background of the Study: Tropical plants of the *Ficus* genus (Moraceae) are among the earliest fruit trees that humans have cultivated. Since ancient times, many folk medicines have used species of this genus to treat a variety of ailments. Evidence from earlier investigations has shown these plants contain abundant secondary metabolites with a variety of structural properties and biological functions.

Place and Duration of Study: The research was carried out at the University of Nairobi (Faculty of Science and Technology, Department of Chemistry) from January to June 2022.

Aim: The study focuses on isolating and identifying secondary metabolites from the stem bark of *Ficus thonningii* Blume found in Kenya and their chemotaxonomic significance.

Methodology: Dried powdered stem bark of *Ficus thonningii* was extracted by maceration at room temperature using CH_2Cl_2/CH_3OH(1:1) to yield a crude extract which was fractionated in a chromatographic column (CC) using silica gel (60 – 120 mesh) as an adsorbent eluting with EtOAc/*n*-hexane followed by CH_3OH/EtOAc. The fractions were purified using silica gel (70 – 230, 230 – 400 mesh) CC and chromatotron eluting with solvents of different polarity, as well as a crystallization technique. Structures of the isolated compounds were elucidated and identified using the spectroscopic method (NMR (1D and 2D)) and by comparison with reported literature data.

Results: Phytochemical investigation of stem bark of *Ficus thonningii*afforded seven compounds, including yukovanol (**1**), 5,7,4′-trihydroxy-3′-(2-hydroxy-3-methyl-3-butenyl)isoflavone (**2**), cajanin (**3**), taxifolin (**4**), protocatechuic acid (**5**), saccharose (**6**), and stigmasterol (**7**). Compounds **1 - 3**, **5** and **7** were not reported from *F. thonningii* until now. Further, compound **6** is being isolated from the genus *Ficus* for the first time.

Conclusion: The chemotaxonomic significance of the isolated phytochemicals demonstrates the taxonomic position of *F. thonningii* in the genus *Ficus* and explains its multiple ethnomedicinal applications.

Keywords: *Ficus thonningii*, Moraceae, Flavonoids, Phenolic acid, Chemotaxonomy

1. INTRODUCTION

The genus *Ficus*(Moraceae) consists of over 850 species widely distributed in tropical and subtropical countries across the globe [1–3]. *Ficus* is among the leading diversified plant genera including creepers, climbers, and stranglers. It also has free-standing deciduous and evergreen trees.*Ficus* species are distinguished by their distinctive syconium-like inflorescence and symbiotic connection with Agaonidae wasps, which pollinate their species exclusively [4,5]. In the African region, 112 species of *Ficus* are recognized currently [6], with 37 being distributed in Kenya within 0 – 2300 m altitude, including *Ficusthonningii*[7,8].

Ficus thonningii(commonly called 'Mugumo' by Kikuyu in Kenya) has a dense, rounded to spreading crown, often epiphytically initially, and is multi-stemmed, evergreen, or short deciduous. The shiny green leaves of *F. thonningii* are alternate, oval (up to 12 cm) with rounded tip and tapering base, whereas the young leaves are pale and finely hairy. The ariel roots are frequently present, and the bark is grey, thin, and smooth [8,9]. *Ficus* species such as *Ficus thonningii* have long been used in indigenous medical systems to treat diarrhea, gonorrhea, diabetes mellitus, inflammation, and induce lactation [10–13].

Phytochemical investigations on the genus *Ficus* have revealed flavonoids (especially isoflavones) [14–18] and terpenoids [19–25] as the major chemical constituents. Phytochemicals such as alkaloids [26–28], coumarins [29–31], and phenolic acids [32,33] have also been reported from various species of *Ficus.* However, the systematic phytochemical studies of *F. thonningii* from East Africa has hitherto not been reported. Therefore, we herein report the isolation and identification of phytoconstituents from the stem bark of *F. thonningii* found in Kenya, and their chemotaxonomic significance.

2. MATERIALS AND METHODS

2.1 Plant collection

The stem bark of *Ficusthonningii*(Fig. 1)was collected from the Riverside drive in September 2020 (1°16'19.2"S 36°48'07.6"E) in Nairobi County, Kenya. The plant was identified by Mr. Patrick C. Mutiso, a taxonomist from the Faculty of Science and Technology (FST), University of Nairobi, Kenya, where a voucher specimen (HIUON 2021/004) was deposited.The stem bark sample was air-dried under shade, powdered, weighed, and stored for subsequent use.

a **b**

Figure 1: (a) *Ficus thonningii* Blume tree and (b) Stem barks of *F. thonningii*

2.2Experimental Procedures

Silica gel 60 – 120, 70 – 230, and 230 – 400 meshes as solid phases for column chromatography (CC), and Sephadex LH–20 (25–100 µm, Sigma Aldrich) were used. Thin Layer Chromatography (TLC) was carried out on pre-coated silica gel 60 plates (0.25 mm; Merck, Darmstadt, Germany). Compounds were visualized under UV light and further by spraying with H_2SO_4–H_2O (5 %, v/v). NMR

spectra were performed on Bruker Advance Neo 500 MHz spectrometer using standard pulse sequences and referenced to residual solvent signals.

2.3Extraction and isolation

Dried powdered stem bark of *Ficus thonningii* (1.7 Kg) was extracted at room temperature withCH_2Cl_2/CH_3OH(1:1, 6 L, 24 h × 3) by maceration to afford 85.7 g of crude extract.80 g of the stem bark crude extract was fractionated in a chromatographic column using silica gel (60 – 120 mesh) as an adsorbent eluting with EtOAc/*n*-hexane (0:10, 1:9, 1.5:8.5, 2:8, 3:7, 3.5:6.5; 4:6, 4.5:5.5, 1:1 and 10:0) followed by CH_3OH/EtOAc (1:9 and 2:8) to yield twelve fractions (HIF_{A-L}).Fraction HIF_I (2.8 g) was subjected to silica gel (70 – 230 mesh) CC eluting with EtOAc/*n*-hexane (1:9 to 10:0), resulting in the isolation of compounds **1**(5.1 mg) and **2** (3.0 mg). Purification of fraction HIF_J (2.81 g) using silica gel (70 – 230 mesh) CC eluting with a gradient ofEtOAc/*n*-hexane (0.5:9.5 to 10:0), resulted in 85 fractions of 30 mL each. The fractions were pooled based on their TLC profiles into two main subfractions (HIF_{J1-2}).

Subfraction HIF_{J1} (700 mg) was purified on a silica gel (70 -230 mesh) CC eluting with a gradient polarity of EtOAc/*n*-hexane (0:10 to 1.5:8.5) to afford a semi-pure compound. The semi-pure compound was purified on a chromatotron using a gradient of EtOAc/*n*-hexane (2:8 to 10:0) to yield compound **3** (13.4 mg). Subfraction HIF_{J2} (900 mg) was also purified using silica gel (230 – 400 mesh) CC eluting with a gradient of EtOAc/*n*-hexane (1:9 to 6:4) to afford compounds **4** (1.06 mg) and **5** (2.82 mg). Fraction HIF_L (3.5 g) afforded brown crystals which, after filtration and recrystallization in CH_3OH, compound **6** (43.2 mg) was obtained. Similarly, compound **7** (33.6 mg) was crystallized in fraction HIF_F (1.02 g), and the crystals were repeatedly washed with *n*-hexane to obtain the compound.

3. RESULTS

Based on the NMR (1D and 2D) and in comparison with the previously published data, the isolated compounds were identified as yukovanol (**1**) [34,35], 5,7,4′-trihydroxy-3′-(2-hydroxy-3- methyl-3-butenyl)isoflavone (**2**) [36], cajanin (**3**) [37], taxifolin (**4**) [38], protocatechuic acid (**5**) [39], saccharose (**6**) [40], and stigmasterol (**7**)[41], (Fig. 2). The NMR data of the isolated compounds are presented below.

Yukovanol (**1**): Yellow powder; ^{1}H NMR (CD_3OD, 500 MHz): *δ*(ppm) 5.02 (1H, d, *J* = 11.6 Hz, H-2), 4.60 (1H, d, *J* = 11.6 Hz, H-3), 5.91 (1H, s, H-6), 7.37 (2H, d, *J* = 8.6 Hz, H-2′/6′), 6.85 (2H, d, *J* = 8.6 Hz, H-3′/5′), 6.62 (1H, d, *J* = 10.1 Hz, H-4″), 5.62 (1H, d, *J* = 10.1 Hz, H-5″), 1.44 (6H, d, *J* = 2.4 Hz, H-7″/8″); ^{13}C NMR (CD_3OD, 125 MHz) *δ*: 85.1 (CH, C-2), 73.7 (CH, C-3), 199.2 (C, C-4), 102.4 (C, C-4a), 163.9 (C, C-5), 97.1 (CH, C-6), 163.6 (C, C-7), 104.1 (C, C-8), 159.2 (C, C-8a), 129.1 (C, C-1′), 130.4 (CH, C-2′/6′), 116.2 (CH, C-3′/5′), 159.3 (C, C-4′), 116.0 (CH, C-4″), 127.7 (CH, C-5″), 79.5 (C, C-6″), 28.6 (CH_3, C-7″), 28.5 (CH_3, C-8″) [34,35].

5,7,4′-trihydroxy-3′-(2-hydroxy-3-methyl-3-butenyl)isoflavone (**2**): Yellow powder; ^{1}H NMR (CD_3OD, 500 MHz): *δ*(ppm) 7.87 (1H, s, H-2), 6.28 (1H, d, *J* = 2.2 Hz, H-6), 6.37 (1H, d, *J* = 2.2 Hz, H-8), 7.28 (1H, dd, *J* = 8.3, 2.3 Hz, H-2′), 6.94 (1H, d, *J* = 8.3 Hz, H-3′), 7.23 (1H, d, *J* = 2.3 Hz, H-6′), 2.86 (1H, dd, *J* = 14.7, 2.3 Hz, H-1a″), 2.99 (1H, dd, *J* = 14.7, 8.77 Hz, H-1b″), 4.44 (1H, m, H-2″), 4.89 (2H, s, H-4″), 1.83 (3H, s, H-5″); ^{13}C NMR (CD_3OD, 125 MHz): *δ*(ppm) 153.0 (CH, C-2), 123.5 (C, C-3), 180.9 (C, C-4), 106.2 (C, C-4a), 162.7 (C, C-5), 99.6 (CH, C-6), 163.4 (C, C-7), 94.3 (CH, C-8), 158.2 (C, C-8a), 123.0 (C, C-1′), 129.4 (CH, C-2′), 117.5 (CH, C-3′), 156.7 (C, C-4′), 126.2 (C, C-5′), 132.5 (CH, C-6′), 38.4 (CH_2, C-1″), 78.4 (CH, C-2″), 147.2 (C, C-3″), 111 (CH_2, C-4″), 18.4 (CH_3, C-5″) [36].

Cajanin (**3**): Pale yellow powder; ^{1}H NMR (CD_3OD, 500 MHz): δ (ppm)8.07 (1H, s, H-2), 6.38 (1H, m, H-6), 6.57 (1H, d, *J*= 2.3 Hz, H-8), 3.89 (3H, s, 7-OCH_3), 6.40 (1H, m, H-3′), 6.36 (1H, m, H-5′), 7.05 (1H, d, *J*= 8.2 Hz, H-6′);^{13}C NMR (CD_3OD, 125 MHz): δ (ppm)157.8 (CH, C-2), 122.8 (C, C-3), 182.8 (C, C-4), 107.1 (C, C-4a), 163.3 (C, C-5), 99.3 (CH, C-6), 167.3 (CH, C-7), 93.2 (CH, C-8), 159.7 (C, C-8a), 56.5 (CH_3, 7-OCH_3), 110.6 (C, C-1′), 157.0 (C, C-2′), 104.2 (CH, C-3′), 160.3 (C, C-4′), 108.1 (CH, C-5′), 133.2 (CH, C-6′) [37].

Taxifolin (**4**): Yellow solid; ^{1}H NMR (CD_3OD, 500 MHz): *δ*(ppm) 4.91 (1H, d, *J* = 11.6 Hz, H-2), 4.50 (1H, d, *J* = 11.6 Hz, H-3), 5.92 (1H, d, *J* = 2.1 Hz, H-6), 5.88 (1H, d, *J* = 2.1 Hz, H-8), 6.85 (1H, dd, *J* = 8.1, 2.1 Hz, H-2′), 6.80 (1H, d, *J* = 8.1 Hz, H-3′), 6.96 (1H, d, *J* = 2.0 Hz, H-6′); ^{13}C NMR (CD_3OD, 125 MHz): *δ*(ppm) 85.1 (CH, C-2), 73.7 (CH, C-3), 198.4 (C, C-4), 101.8 (C, C-4a), 164.5 (C, C-5), 97.4 (CH, C-6), 165.3 (C, C-7), 96.3 (CH, C-8), 164.5 (C, C-8a), 129.9 (C, C-1′), 120.9 (CH, C-2′), 116.1 (CH, C-3′), 147.2 (C, C-4′), 146.3 (C, C-5′), 115.9 (CH, C-6′) [38].

Protocatechuic acid (**5**): White powder; ^{1}H NMR (CD_3OD, 500 MHz):*δ*(ppm) 7.43 (1H, d, *J* = 1.9 Hz, H-2), 6.77 (1H, d, *J* = 8.2 Hz, H-5), 7.41 (1H, dd, *J* = 8.2, 1.9 Hz, H-6); ^{13}C NMR (CD_3OD, 125 MHz): *δ*(ppm) 125.0 (C, C-1), 117.7 (CH, C-2), 145.9 (C, C-3), 150.9 (C, C-4), 115.6 (CH, C-5), 123.7 (CH, C-6), 170.0 (CO) [39].

Saccharose (**6**): Colorless crystals; ^{1}H NMR (D_2O, 500 MHz): *δ*(ppm) 5.40 (1H, d, *J* = 3.8 Hz, H-1), 3.54 (1H, dd, *J* = 10.0, 3.8 Hz, H-2), 3.75 (1H, t, *J* = 10.0, 9.1 Hz, H-3), 3.46 (1H, t, *J* = 9.5 Hz, H-4), 3.83 (1H, m, H-5), 3.80 (2H, m, H-6), 3.66 (2H, s, H-1′), 4.20 (1H, d, *J* = 8.8 Hz, H-3′), 4.04 (1H, t, *J* = 8.6 Hz, H-4′), 3.87 (1H, m, H-5′), 3.80 (2H, m, H-6′); ^{13}C NMR (CD_3OD, 125 MHz): *δ* (ppm) 92.1 (CH, C-1), 71.0 (CH, C-2), 72.5 (CH, C-3), 69.1 (CH, C-4), 72.3 (CH, C-5), 60.0 (CH_2, C-6), 61.2 (CH_2, C-1′), 103.6 (C, C-2′), 76.3 (CH, C-3′), 73.9 (CH, C-4′), 81.3 (CH, C-5′), 62.3 (CH_2, C-6′) [40].

Stigmasterol (**7**): White amorphous solid;^{1}H NMR ($CDCl_3$, 500 MHz): δ (ppm) 1.84 (2H, m, H-1), 1.99 (2H, m, H-2), 3.52 (1H, tt, *J* = 11.6, 4.9 Hz, H-3), 2.28 (2H, m, H-4), 5.34 (1H, dt, *J* = 5.5, 1.9 Hz, H-6), 1.50 (2H, m, H-7), 1.43 (1H, m, H-8), 0.92 (1H, m, H-9), 1.47 (2H, m, H-11), 2.01 (2H, m, H-12), 0.99 (1H, m, H-14), 1.84 (2H, m, H-15), 1.25 (2H, m, H-16), 1.10 (2H, m, H-17), 0.68 (3H, s, H-18), 1.00 (3H, s, H-19), 2.03 (1H, m, H-20), 1.02 (3H, d, *J* = 6.7 Hz, H-21), 5.14 (1H, dd, *J* = 15.1, 8.6 Hz, H-22), 5.02 (1H, dd, *J* = 15.1, 8.6 Hz, H-23), 1.52 (1H, m, H-24), 1.46 (1H, m, H-25), 0.92 (3H, d, *J* = 6.4), 0.81 (3H, dd, *J* = 8.3, 6.8 Hz, H-27), 1.60 (2H, m, H-28), 0.84 (3H, dd, *J* = 8.3, 6.8 Hz, H-29); ^{13}C NMR (CD_3OD, 125 MHz): *δ* (ppm) 37.4 (CH_2, C-1), 31.8 (CH_2, C-2), 72.0 (CH, C-3), 42.4 (CH_2, C-4), 140.9 (C, C-5), 121.9 (CH, C-6), 31.8 (CH_2, C-7), 32.1 (CH, C-8), 50.3 (CH, C-9), 36.6 (C, C-10), 21.2 (CH_2, C-11), 39.9 (CH_2, C-12), 42.5 (C, C-13), 56.9 (CH, C-14), 24.4 (CH_2, C-15), 29.9 (CH_2, C-16), 56.2 (CH, C-17), 12.0 (CH_3, C-18), 19.5 (CH_3, C-19), 40.6 (CH, C-20), 21.4 (CH_3, C-21), 138.5 (CH, C-22), 129.4 (CH, C-23), 51.4 (CH, C-24), 32.0 (CH, C-25), 18.9 (CH_3, C-26), 19.2 (CH_3, C-27), 26.2 (CH_2, C-28), 12.1 (CH_3, C-29)[41].

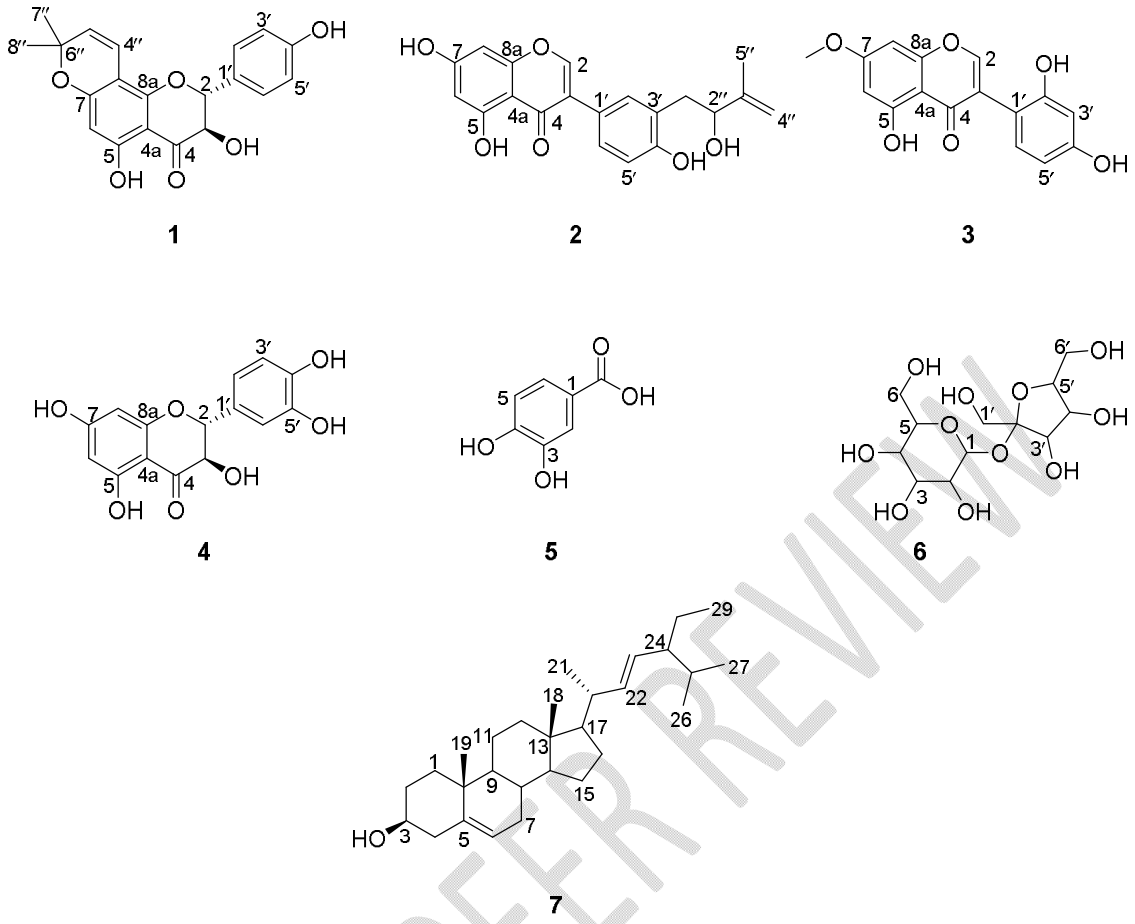

Figure2:Structures of compounds **1** – **7**.

4. DISCUSSION

The present phytochemical investigation led to the isolation of seven compounds from the stem bark of *F. thonningii*, including four flavonoids (**1** – **4**), one phenolic acid (**5**), one sugar (**6**), and a sterol (**7**). Compounds **6** was isolated from the genus *Ficus* for the first time, while this is the first report of compounds **1** – **2** and **4** – **7** from *F. thonningii*. Therefore, these findings improve the *F. thonningii*'s chemical profile and provide additional data for the chemotaxonomic research of the genus *Ficus*.

The presence of flavonoid derivatives (compounds **1** – **4**) might perhaps explain some of the traditional uses of this *Ficus* species. For instance, Compound **1** (yukovanol) was found to have a potent Effective at initiating cell cycle arrest and promoting osteoblast proliferation, cajanin (compound **3**) is a potent antimelanogenic drug. It also significantly increased bone mineral density and strength [42,43]. Furthermore, compound **4** (taxifolin) has been shown to exhibit anti-

inflammatory effects, plasma cholesterol-lowering effects, anticarcinogenic, hepatoprotective, and antiviral activities [44,45]. Compound **5** (protocatechuic acid), a phenolic acid, is endowed with intriguing biological properties. These properties include anti-bacterial, antioxidant, antidiabetic, anticancer, and anti-ageing [46–48].For the synthesis of organic compounds, such as 1,4-dihydropyrano[2,3-c]pyrazole and *β*-aminoketone derivatives with intriguing pharmacological activities, saccharose (compound **6**) has been frequently used as an effective and homogeneous green catalyst[49,50].One phytochemical component that has been isolated from numerous plants and studied for a variety of pharmacological and biological properties is stigmasterol (compound **7**). Numerous prior studies indicate that stigmasterol has potent antibacterial, antioxidant, anticancer, anti-inflammatory, analgesic, cardiovascular, and antifungal properties [51–53]. As a result, the isolated phytochemicals may be used as lead molecules in the development of new therapeutics.

Whereas, compound **1,** which has only been reported previously from *F. tikoua* [54]*,* in this study it has been isolated in the genus *Ficus* and Moraceae family for the second time, revealing a close phylogenetic relationship between *F. thonningii* and *F. tikoua.* As a result, compound **1** might be used as a chemotaxonomic marker to distinguish the genus *Ficus*. In addition, compound **1** (which its occurrence is very rare) was also reported from other species of *Desmodium*, such as *Desmodium caudatum*[55] and *Desmodium triquetrum*[56], which demonstrated that the two genera (*Ficus* and *Desmodium*) might possibly be related. Furthermore, Compound **6** is yet to be reported from other species of *Ficus;* as such, it can act together with compound **1** aschemotaxonomic markers for *F. thonningii*.

Isoflavones are the major class of flavonoids reported from *Ficus* species [14,16,54]; therefore, the isolation of compounds **2** – **3** agrees with the previous reports and supports the taxonomic position of *F. thonningii* in the genus. Compounds **2** and **3** were previously isolated from *F. pumila*[57], *F. nervosa*[14], and *F. ovata* [58].The current findings indicate chemotaxonomic relationships between *F. thonningii* and these species. Compound **5** was reported fromthe fruits of *F. aurata* and *F. hispida*[32], leaves of *F. trigonata* [59], and stem bark of *F. pandurate* [22], indicating the chemotaxonomic relationship between *F. thonningii* and the other species. Taxifolin (**4**) and stigmasterol (**7**) are often present in plants and so have a less taxonomic importance.

4. CONCLUSION

In conclusion, our study provides new phytochemical information for *F. thonningii* and revealed that *F. thonningii* shares certain flavonoids and phenolic acids with other *Ficus* species such as *F. hipida, F. tikoua, F. pumila, F. tsiangii, F. ovata,F. aurata* and *F. nymphaefolia* supporting its taxonomic assignment to the genus *Ficus*. The isolated compounds in this work can potentially be chemotaxonomic markers for *F. thonningii*. Our findings have increased the chemical diversity of *F. thonningii* compounds and demonstrated their chemotaxonomic relevance. Besides, the isolation of different classes of secondary metabolites that possess diverse pharmacological potentials from *F. thonningii*explained its multiple ethnomedicinal applications.

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