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Further saponins from *Fagonia cretica*

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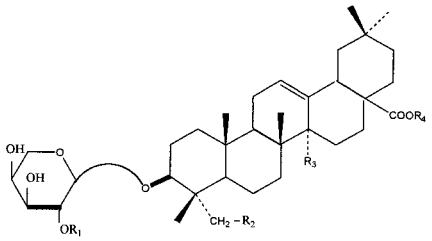
Three triterpenoid saponins including two new ones were isolated and identified from the aerial parts of *Fagonia cretica*. The new saponins were characterized as 3-*O*-[β-D-xylopyranosyl(1→2)α-L-arabinopyranosyl]27-hydroxyoleanolic acid 28-*O*-[β-D-glucopyranosyl(1→6)β-D-glucopyranosyl]ester and 3β-*O*-[β-D-xylopyranosyl(1→2)α-L-arabinopyranosyl]olean-12-en-27-al-28-oic acid 28-*O*-[β-D-glucopyranosyl(1→6)β-D-glycopyranosyl] ester. The structures were determined by spectral analyses. The NMR assignments were made by means of HOHAHA, ^{1}H-^{1}H COSY, HMQC, HMBC spectra and NOE studies.

1. Introduction

Saponins constitute a group of natural products with a wide range of biological activities [1–4]. The pharmacological importance associated with these compounds has encouraged us to examine their natural occurrence in *Fagonia cretica* (Zygophylaceae). This plant is a shrub widely distributed in Egypt and Pakistan and used in the traditional system of medicine, like other *Fagonia* plants, as a remedy for skin lesions. Our pharmacological investigation, on the saponin-containing fraction from *F. cretica*, showed that it exhibited significant anti-inflammatory activity and considerable analgesic and antipyretic effects [5]. Phytochemical investigation has resulted in the isolation of the triterpenes oleanolic acid and hederagenin as well as seven bisdesmosides of oleanane type triterpene acid [6, 7]. In continuation of our work on the saponin fraction from *F. cretica*, we report here the isolation of three triterpenoid saponins **1**–**3** including two new ones (**2** and **3**). The saponins were isolated and purified by the combined use of CC and HPLC techniques.



	R_1	R_2	R_3	R_4
1-	Xyl	OH	CH_3	Glc (1 → 6) Glc
2-	Xyl	H	CH_2 – OH	Glc (1 → 6) Glc
3-	Xyl	H	CHO	Glc (1 → 6) Glc

Glc = β– D– glucopyranosyl
Xyl = β – D – xylopyranosyl

The identification of the saponin structures in general and that of the sugar components in particular was permitted by the use of positive ion FAB mass spectrum, and 1D and 2D NMR techniques. ^{1}H-^{1}H COSY, HOHAHA and HMQC spectra established the structure of the monosaccharide units while NOE difference measurements and HMBC spectrum determined the interglycosidic linkages and the attachment of the sugar chain to the aglycone. ^{1}H and ^{13}C NMR signal assignments were made possible by the combined use of ^{1}H-^{1}H COSY, HMQC and HMBC experiments. The relative orientation of the hydrogen atoms was deduced from the coupling constant values taken from the 1D spectrum or their relative magnitudes from the shape of the cross peaks in the ^{1}H-^{1}H COSY spectrum. The NMR data of saponins **2** and **3** are shown in Tables 1 and 2.

2. Investigations, results and discussion

The FAB MS of saponin **2** exhibited a $[M + Na]^+$ ion at m/z 1083 $[C_{52}H_{84}O_{22} + Na]^+$. Upon acid hydrolysis, **2** yielded sugar components identified by GC analysis as D-glucose, D-xylose and L-arabinose. The ^{1}H NMR spectrum showed the typical signals of a triterpene acid moiety of oleanane type, except the lower field positions of signals due to H-12 (δ 5.78) and H-18 (δ 3.28). The six-methyl signals at δ 1.20, 1.06, 0.92, 1.11, 0.83 and 0.90 were assigned by the HMBC spectrum for H_3-23, H_3-24, H_3-25, H_3-26, H_3-29 and H_3-30. These signals were correlated in the HMQC spectrum with their corresponding carbons at δ 27.9, 16.4, 16.1, 19.0, 33.1 and 23.9. The hydroxy methylene signals at δ 3.76 and 4.03 were assigned for H_2-27 on the basis of their correlations with the signal at 23.9 (C-15) in the HMBC spectrum and the signal at δ 64.5 (C-27) in the HMQ spectrum. These findings together with the observed unusual shift of the olefinic carbons at δ 128.0 and 139.2, in the ^{13}C NMR spectrum, allowed identification of the aglycone moiety as 27-hydroxy oleanolic acid, the structural isomer of hederagenin. This conclusion was confirmed from the similarity between its ^{13}C NMR data and the corresponding data of saponins bearing the same moiety [8, 9]. Considering the δ values of the signals due to C-3 (88.9 ppm) and C-28 (176.6 ppm), saponin **2** is a 3,28 bisdesmoside. Alkaline hydrolysis of **2** yielded D-glucose as the sole sugar component. In the ^{1}H NMR spectrum, four anomeric proton signals were observed at δ 4.83 (d, J = 6.6 Hz), 5.06 (d, J = 6.6 Hz), 6.26 (d, J = 7.8 Hz) and 5.02 (d, J = 7.8 Hz) and assigned for one α-arabinose, one β-xylose and two β-glucose units respectively. The proton system of each unit was analyzed using the HOHAHA difference method and the sequence of protons was then deduced from the ^{1}H-^{1}H COSY spectrum. The HMQC spectrum correlated all proton resonances with their corresponding carbons in each sugar unit. Comparison of ^{13}C assignments with those of reference methyl glycosides revealed the presence of a terminal β-D-glucopyranosyl unit, a terminal β-D-xylopyranosyl unit, a 2-substituted α-L-arabinopyranosyl unit and a 6-substituted β-D-glucopyranosyl unit. Information about the sequence of the sugar units and the

linkage sites to the aglycone was obtained fron the NOE studies as well as by coupling between carbons and protons of the neighbouring residues in the HMBC study. In the NOE difference measurements, when the anomeric proton signal of xylose (δ 5.06) was irradiated, the H-2 signal of arabinose (δ 4.49) responded. Likewise, the signals of H-6 protons of the inner glucose at δ 4.34 and 4.70 responded on irradiation of the anomeric proton sig-

Table 1: ^{1}H NMR spectral data of compounds 1–3 in pyridine d_5

H	1	2	3
Aglycone moiety			
1 ax.	1.05	0.98 (dt, 13.0, 3.0)	0.89
1 eq.	1.58 (brd, 14.7)	1.50	1.53
2 ax.	2.02	1.85	1.82
2 eq.	2.22	2.06	2.10
3	4.25	3.15 (dd, 10.0, 4.0)	3.16 (dd, 10.7, 4.7)
5	1.70	0.87	0.62 (brd, 9.3)
6 ax.	1.41 (brt, 13.1)	1.37	1.23
6 eq.	1.71	1.51	1.42 (brd, 6.7)
7 ax.	1.60 (brt, 14.7)	1.85	1.56
7 eq.	1.30 (brd, 14.1)	1.40 (brd, 13.0)	1.23
9	1.76	2.19 (brt, 10.0)	1.75 (dd, 9.3, 6.7)
11 a, e	1.94	1.94	2.08
12	5.41 (t, 3.0)	5.78 (t, 3.0)	5.94 (t, 3.0)
15 ax.	2.29 (dt, 13.9, 3.5)	2.31 (dt, 14.0, 4.0)	2.17
15 eq.	1.08	1.45	2.28 (brd, 13.3)
16 ax.	2.02	2.10 (dt, 14.0, 4.0)	2.16
16 eq.	1.90	1.95	1.98
18	3.19 (dd, 13.9, 3.5)	3.28 (dd, 13.0, 4.0)	3.22 (dd, 14.0, 4.7)
19 ax.	1.72	1.71	1.20
19 eq.	1.25	1.30	1.08
21 ax.	1.31	1.28	1.19
21 eq.	1.08	1.10	1.02
22 ax.	1.91	1.93	1.84 (dt, 14.7, 3.4)
22 eq.	1.77	1.75 (brd, 13.0)	1.69 (brd, 14.4)
23	3.69 (d, 10.9), 4.26	1.20 (s)	1.12 (s)
24	1.03 (s)	1.06 (s)	1.00 (s)
25	1.01 (s)	0.92 (s)	0.87 (s)
26	1.13 (s)	1.11 (s)	1.10 (s)
27	1.18 (s)	3.76 (d, 10.0), 4.03	9.90 (s)
29	0.87 (s)	0.83 (s)	0.75 (s)
30	0.89 (s)	0.90 (s)	0.85 (s)
C-3 sugar			
Arabinose			
1	5.08 (d, 6.9)	4.83 (d, 6.6)	4.82 (d, 6.7)
2	4.49 (t, 6.9)	4.49 (t, 6.6)	4.49 (t, 6.7)
3	4.14	4.28	4.28
4	4.24 (m)	4.32	4.31
5	3.63 (d, 10.9), 4.23	3.79 (d, 11.0), 4.28	3.77 (d, 10.7), 4.27
Xylose			
1	5.06 (d, 6.9)	5.06 (d, 6.6)	5.07 (d, 6.7)
2	4.06	4.06	4.06
3	4.08	4.07	4.07
4	4.15	4.18	4.18
5	3.57 (t, 10.9), 4.30	3.58 (t, 10.0), 4.32	3.58 (t, 10.7), 4.31
C-28 sugar			
Inner glucose			
1	6.23 (d, 7.8)	6.26 (d, 7.8)	6.26 (d, 7.8)
2	4.10 (t, 7.8)	4.14 (t, 7.8)	4.12 (t, 7.8)
3	4.19 (t, 8.0)	4.19 (t, 8.0)	4.19 (t, 8.0)
4	4.30 (t, 8.0)	4.30 (t, 8.0)	4.31 (t, 8.0)
5	4.10	4.12	4.13
6	4.34 (dd, 12.0, 3.5)	4.34 (dd, 12.0, 3.5)	4.34 (dd, 12.0, 3.5)
6	4.69 (d, 12.0)	4.70 (d, 12.0)	4.72 (d, 12.0)
Terminal glucose			
1	5.02 (d, 7.8)	5.02 (d, 7.8)	5.02 (d, 7.8)
2	3.98 (t, 7.8)	3.99 (t, 7.8)	3.99 (t, 7.8)
3	4.16	4.18	4.18
4	4.18	4.20	4.19
5	3.87 (m)	3.87 (m)	3.88 (m)
6	4.33 (dd, 12.0, 4.0)	4.33 (dd, 12.0, 3.5)	4.35 (dd, 12.0, 4.0)
6	4.46 (d, 12.0)	4.46 (d, 12.0)	4.47 (d, 12.0)

Arabinose = α-L-arabinopyranosol
Xylose = β-D-xylopyranosyl
Glucose = β-D-glucopyranosyl

Table 2: ^{13}C NMR spectral data of compounds 1–3 in pyridine d_5

Carbon	1	2	3
Aglycone moiety			
1	39.0	38.8	38.6
2	26.3	26.6	26.5
3	81.4	88.9	88.5
4	43.7	39.6	39.6
5	47.4	55.9	55.8
6	18.2	18.7	18.4
7	32.9	33.7	35.7
8	40.0	40.7	41.9
9	48.3	48.7	49.8
10	36.9	37.2	37.4
11	23.9	24.4	23.9
12	123.0	128.0	128.4
13	144.2	139.2	136.9
14	42.2	48.0	59.1
15	28.4	23.9	20.9
16	23.4	23.5	23.4
17	47.1	46.9	46.7
18	41.7	41.5	42.2
19	46.3	45.4	43.6
20	30.8	30.8	30.6
21	34.0	33.9	33.6
22	32.6	32.6	32.0
23	63.8	27.9	27.7
24	13.2	16.4	16.4
25	16.3	16.1	16.3
26	17.6	19.0	18.4
27	26.1	64.5	207.3
28	176.5	176.6	176.3
29	33.1	33.1	32.9
30	23.7	23.9	23.9
C-3-sugar			
Arabinose			
1	104.4	105.1	105.1
2	82.0	81.5	81.6
3	74.0	73.8	73.8
4	68.8	68.6	68.6
5	65.9	65.6	65.6
Xylose			
1	106.8	106.7	106.8
2	76.1	76.1	76.1
3	78.3	78.2	78.3
4	71.0	71.1	71.1
5	67.4	67.4	67.4
C-28-sugar			
Inner glucose			
1	95.7	95.8	95.8
2	74.0	73.9	74.0
3	78.8	78.8	78.8
4	71.0	71.1	71.1
5	78.0	78.0	78.1
6	69.5	69.5	69.6
Terminal glucose			
1	105.3	105.3	105.3
2	75.2	75.2	75.2
3	78.4	78.4	78.5
4	71.6	71.7	71.7
5	78.4	78.4	78.5
6	62.7	62.8	62.8

Arabinose = α-L-arabinopyranosol
Xylose = β-D-xylopyranosyl
Glucose = β-D-glucopyranosyl

nal of the terminal glucose at δ 5.02. Also the signal due to H-3 aglycone at δ 3.15 responded when the anomeric proton signal of arabinose at δ 4.83 was irradiated. In the HMBC spectrum, the observed correlations between protons/carbons were H-1 of xylose (δ 5.06)/C-2 of arabinose (δ 81.5); H-1 of terminal glucose (δ 5.02)/C-6 of inner glucose (δ 69.5); H-1 of arabinose (δ 4.83)/C-3 aglycone (δ 88.9) and H-1 of inner glucose (δ 6.26)/C-28 aglycone (δ 176.6). Therefore saponin **2** possessed a disaccharide chain composed of two (1→6) linked glucose units bonded to C-28 carbonyl group by ester linkage while the remaining sugars, xylose and arabinose, form a (1→2) disaccaride chain bound to the aglycone C-3 by a glycosidic linkage. The similarity between the ^{13}C NMR data due to the sugar portion of **2** and the corresponding data of saponins reported from the same plant which possess a [β-D-xylopyranosyl(1→2)α-L-arabinopyranosyl] unit at C-3 [6] or [β-D-glucopyranosyl(1→6)β-D-glycopyranosyl] chain at C-28 [7], further supported the above sugar chain assignment.

Based on the above studies saponin **2** was assigned the structure of 3-*O*-[β-D-xylopyranosyl(1→ 2)α-L-arabinopyranosyl]27-hydroxy oleanolic acid 28-*O*-[β-D-glucopyranosyl(1→6)β-D-glucopyranosyl] ester. This is the first reported occurrence of this compound in nature.

Saponin **1** showed in its ^{1}H NMR spectrum the characteristic signals of triterpene acid moiety of oleanane type. The assigned carbon signals in the ^{13}C NMR spectrum, due to the aglycone moiety, were in good agreement with those of hederagenin [10]. In the HMBC spectrum, the observed correlations, between the signal due to C-24 at δ 13.2 and two signals attributable to H_2-23 at δ 3.69 (d, J = 10.9) and δ 4.26, supported the aglycone structure. Upon acid hydrolysis of saponin **1**, the sugar components D-glucose, D-xylose and L-arabinose were released. In the ^{13}C NMR spectrum, the low field position of the aglycone C-3 signal at δ 81.4 and the upfield carbonyl signal at δ 176.5, with respect to the corresponding values of hederagenin, suggested glycosylation of the aglycone moiety at C-3 and C-28. The FAB MS of **1** like that of **2** exhibited a $[M+Na]^+$ ion at m/z 1083 $[C_{52}H_{84}O_{22} + Na]^+$, in addition to a fragment ion at m/z 760 corresponding to $[1083 + H\text{-}2 \text{ hexoses}]^+$. On alkaline hydrolysis of **1**, D-glucose was the sole sugar component released. The observed anomeric proton signals in the ^{1}H NMR spectrum which appeared at δ 5.08 (d, J = 6.9 Hz), 5.06 (d, J = 6.9 Hz), 6.23 (d, J = 7.8 Hz), 5.02 (d, J = 7.8 Hz) and their corresponding carbons at δ 104.4, 106.8, 95.7, 105.3, assigned by considering the HMQC spectrum, were almost identical to those of saponin **2**. The two sugar carbon signals observed at δ 69.5 and 82.0, in the ^{13}C NMR of **1**, were similar to two signals assigned for C-2 of inner arabinose and C-6 of inner glucose in the spectrum of saponin **2**. These spectral observations allowed us to suggest that saponin **1** might have two disaccaride chains identical to those of saponin **2**. Inspection of the HOHAHA and ^{1}H-^{1}H COSY spectra confirmed that **1** and **2** possessed identical monosaccharide units. Irradiation at each anomeric proton signal in the NOE studies and observing cross peaks in the HMBC spectrum verified the resemblance between **1** and **2** in sugar sequence and linkages.

From the above, saponin **1** was formulated as 3-*O*-[β-D-xylopyranosyl(1→2)α-L-arabinopyranosyl]hederagenin 28-*O*-[β-D-glycopyranosyl(1→6)β-D-glycopyransoyl] ester. A compound with identical structure has been reported from *Akebina quinata* [11]. Its structure evaluation was based only on chemical means and no spectral data were provided.

The FAB MS of saponin **3** displayed a $[M + Na]^+$ ion at m/z 1081 $[C_{52}H_{82}O_{22} + Na]^+$; less than that of **2** by 2 mu. The spectrum also showed a fragment ion at m/z 953 corresponding to $[1081\text{-}CO]^+$. The ^{1}H NMR spectrum showed the existence of six methyl singlets at δ 1.12,

1.00, 0.87, 1.10, 0.75 and 0.85 assigned by an HMBC study for H_3-24, H_3-25, H_3-26, H_3-29 and H_3-30 of a triterpene acid moiety with the oleanane skeleton. It also displayed a signal due to H-12 at lower field position (δ 5.94) relative to that of **1**. Furthermore ^{13}C MNR spectrum showed signals due to the double bond at unusual δ values (128.4, 136.9 ppm). It also exhibited in the downfield region two resonances at δ 176.3 and 207.3, assigned for the C-28 ester carbonyl function and the aldehydic carbonyl group at C-14 of the aglycone moiety respectively. The latter assignment was concluded from the observed connectivity in the HMBC spectrum between the signal at δ 207.3 and the signal due to ax. H-15 at δ 2.17 which was in turn showed to correlate with the C-15 signal at δ 20.9 in the HMQC study. These findings together with the observed δ values due to B−D ring carbons with respect to the corresponding values in **1**, allowed identification of the aglycone moiety of saponin **3** as 3β-hydroxy olean-12-en-27-al-28-oic acid previously reported by us for the first time, from the same source [7]. The resonances due to C-3 and C-28 of the aglycone moiety at δ 88.5 and 176.3, suggested glycosylation at these carbons. The acid and alkaline hydrolysis of **3** gave identical sugar components to those obtained from **1** and **2** and the 2D NMR techniques (^{1}H-^{1}H COSY, HOHAHA, HMQC and HMBC) together with NOE measurements proved identical monosaccaride units, sequencing and linkages.

Therefore saponin **3** was deduced to be 3β-*O*-[β-D-xylopyranosyl(1→2)α-L-arabinopyranosyl]olean-12-en-27-al-28-oic acid 28-*O*-[β-D-glucopyranosyl(1→6)β-D-glucopyranosyl] ester. This saponin is a new natural compound and the second reported saponin from *F. cretica* bearing an aglycone possessing an aldehyde function at C-14. To the best of our knowledge, no olean-12-en triterpene derivative, containing a C-14 aldehyde residue, has been isolated from any other plant source.

Inspection of NMR data in Tables 1 and 2 revealed that some resonances due to the aglycone moiety of **1** were modified in **2** and **3**, arising from the introduction of a CH_2OH or CHO moiety respectively instead of CH_3 at C-14. The H-9 resonance was deshielded by 0.43 ppm in **2** and appeared as broad triplet instead of double of doublet whereas it remained unaffected in **3**. The H-12 resonance in **3** was deshielded by about 0.53 ppm compared with 0.37 ppm in case of **2**. The resonance due to the eq. H-15 (α) was less deshielded in **2** (0.37 ppm) compared with **3** (1.20 ppm). Although, the H_2-19 resonances remained more or less unchanged in **2**, the corresponding ones in **3** were shielded by 0.17 (eq.) and 0.52 (ax.) respectively. The resonance due to C-12 in both **2** and **3** was deshielded by about 5.0 ppm whereas the C-13 resonances were shielded by 5.0 and 7.3 ppm respectively. The observed deshielding of C-14 resonance in **2** (5.8 ppm) was less than that in **3** (6.9 ppm) and the shielding of C-15 in **2** was about 4.5 ppm compared with 7.5 ppm for **3**.

3. Experiment

3.1. Equipment

Optical rotations were measured with a JASO DIP-1000 digital polarimeter. JEOL JMS-SX 102 was used for positive mode FAB-MS. ^{1}H and ^{13}C NMR spectra were obtained with a JEOL-α-400 FT-NMR spectrometer (^{1}H-400 MHz; ^{13}C 100 MHz, in pyridine-d_5 at 35 °C) and chemical shifts were given in ppm with TMS as int. standard. HPLC was carried on JASCO System 800 and GC analysis was performed with G-3000 gas chromatography.

3.2. Plant material, extraction and isolation

F. cretica was collected from the Borg-El Arab area, Alexandria, Egypt in March 1990 and identified by Dr. M. El Gibaly at the plant Taxonomy Department, N.R.C, Cairo where a voucher specimen was deposited.

Dried aerial parts (5 kg) were soaked twice in 60% aqueous EtOH at room temperature. The combined extract was evaporated under vacuum and the residue (115 g) was dissolved in MeOH and diluted with Me_2CO to precipitate the crude saponin (48 g). The latter was chromatographed on a silica gel column using a mixture of $CHCl_3$−MeOH then $CHCl_3$−MeOH−H_2O. A part (3.6 g) of the residue of the fraction eluted with $CHCl_3$−MeOH−H_2O (70 : 27 : 3) was passed through a porous polymer gel column (Mitsubishi Diaion HP-20). After washing with water, a methanolic eluate was chromatographed on Develosil ODS-10 (2 cm × 25 cm) using a MeCN−H_2O system to give **1** (30 mg), **2** (32 mg) and **3** (20 mg).

Saponin **1**. Amorphous powder $[\alpha_D^{23}] + 14.8°$ (c = 1.35, MeOH); FABMS (m/z) 1083 $[C_{52}H_{84}O_{22} + Na]^+$, 950, 802, 760, 714, 601; ^{1}H and ^{13}C NMR see Tables 1 and 2.

Saponin **2**. Amorphous powder $[\alpha_D^{23}] + 1.3°$ (c = 1.49, MeOH); FABMS (m/z) 1083 $[C_{52}H_{84}O_{22} + Na]^+$, 840, 810, 752, 620, 460, 437; ^{1}H and ^{13}C NMR see Tables 1 and 2.

Saponin **3**. Amorphous powder $[\alpha_D^{23}] + 65.5°$ (c = 0.33, MeOH); FABMS (m/z) 1081 $[C_{52}H_{82}O_{22} + Na]^+$, 1053, 953, 884, 733, 601, 385; ^{1}H and ^{13}C NMR see Tables 1 and 2.

3.3. General method for acid hydrolysis

Each saponin (1 mg) was heated for 30 min at 100 °C in dioxane (50 μl) and 2 M HCL (50 μl). The reaction mixture was diluted with H_2O and extracted with EtOAc. The monosaccharide units in the aq. layer were analyzed as follows [12]: the aq. layer of the reaction mixture of each saponin was passed through an Amberlite IRA-60E column (6 × 50 mm) and the eluate was concentrated. Each monosaccharide fraction was dissolved in pyridine (25 μl) and stirred with D-cysteine methyl ester (3 mg) for 1.5 h at 60 °C. To the reaction mixture, hexamethyldisilazane (10 μl) and trimethylsilyl chloride (10 μl) were added and the reaction mixture was stirred at 60 °C for 30 min. The supernatant of the mixture was then analyzed by GC. Conditions: column, supelco SPB-TM (0.25 mm × 27 m); column temperature, 215 °C; carrier gas, N_2; retention times, D-arabinose (11.0 min), L-arabinose (11.9 min), D-glucose (21.4 min), L-glucose (20.5 min), D-xylose (11.7 min), L-xylose (11.0 min). From saponins **1**, **2**, **3**, L-arabinose, D-xylose and D-glucose were detected.

3.4. General method for alkaline hydrolysis

A solution of saponin (1 mg) in 5% aq. KOH (3 ml) was heated at 100 °C for 30 min. The reaction mixture was neutralized with 5% HCl and then extracted with EtOAc. The residual aq. layer containing the sugar components was analyzed as shown before.

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