

## OZONOLYSIS OF 11-DESOXYGLYCYRRHETIC ACID AND ITS DERIVATIVES

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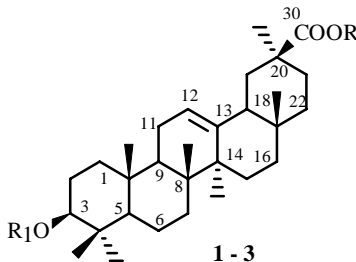
*12-Oxo derivatives of 11-desoxoglycyrrhetic acid and its derivatives were produced by oxidative transformation using ozone. Olean-3,12-dion-30-oic acid was produced for the first time by exhaustive ozonolysis of 11-desoxoglycyrrhetic acid at -60°C.*

**Key words:** 11-desoxoglycyrrhetic acid, ozonolysis.

Oxidative transformations of bioactive plant triterpenoids is of great interest for preparing new oxygenated derivatives, seeking selective oxidizing reagents, and studying structure—activity relationships of derivatives of natural compounds. Introduction of additional oxygenated functional groups into triterpenoids can significantly affect their biological activity [1-3].

Ozone is an effective and ecologically clean oxidant that has been used in various studies to prepare oxygenated derivatives of oleanolic acid and 18 $\beta$ -glycyrrhetic acid (GLA), the principal triterpenoid from licorice roots (*Glycyrrhiza glabra* L. and *G. uralensis* Fisher). It had been thought that the C12=C13 olefinic bond does not react with O<sub>3</sub> due to steric hindrance [4-6]. Prolonged (50 h) ozonation of GLA methyl ester acetate produced the methyl ester of 3-acetoxy-12,13-epoxy-18 $\beta$ -olean-11-on-30-oic acid in 40% yield [5].

The goal of our work was to study transformations of 11-desoxo-GLA (**1**) and its derivatives **2** and **3** that were caused by ozone.

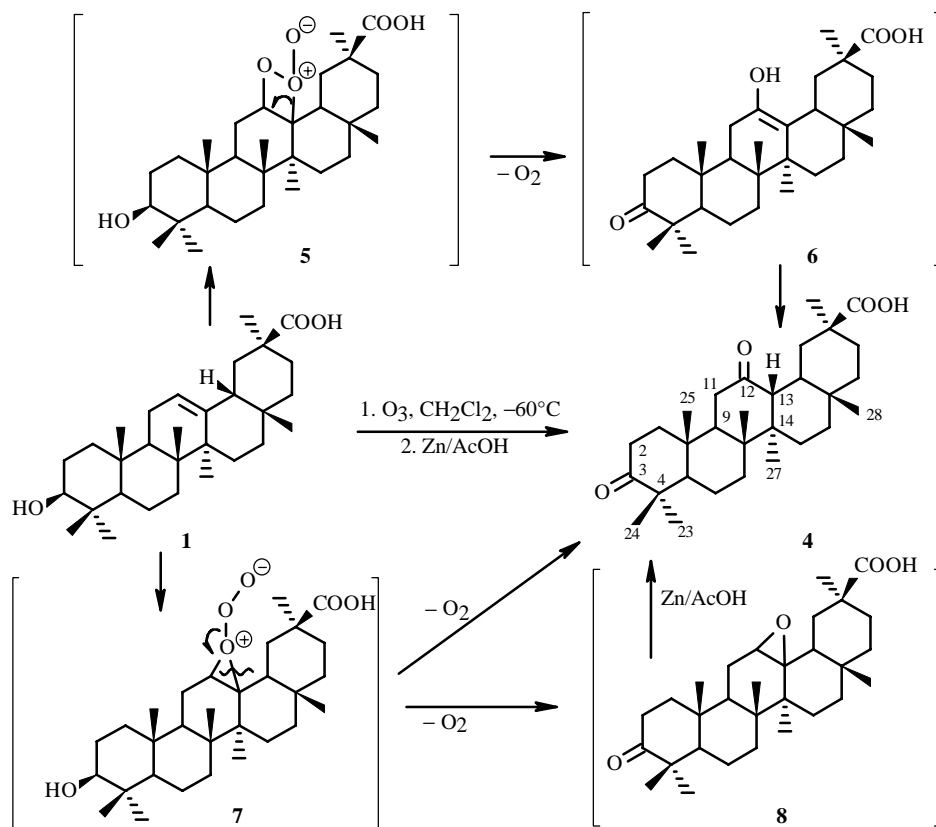


**1:** R = R<sub>1</sub> = H; **2:** R = H, R<sub>1</sub> = Ac; **3:** R = Me, R<sub>1</sub> = H

Ozonolysis of 11-desoxo-GLA (**1**) in CH<sub>2</sub>Cl<sub>2</sub> at -60°C until ozone appeared at the outlet from the flask (until the solution turned blue) and subsequent reduction of the reaction products with Zn powder in CH<sub>3</sub>COOH for 1 h at 0°C produced a mixture of products that was separated by column chromatography (CC) over silica gel (SG). Compound **4** (36% yield), which was identified by its PMR and <sup>13</sup>C NMR spectra as olean-3,12-dion-30-oic acid (Scheme 1), and starting **1**, the TLC and PMR spectrum of which agreed with a known sample, were isolated pure from the oxidation products.

The PMR spectrum of **4** had seven singlets for methyls and a resonance for proton  $\beta$ -H13 at 2.8 ppm with spin—spin coupling constant 4 Hz. This was typical of derivatives of  $\beta$ -amyrin triterpenoids with oxygenated functional groups on C12 [4, 7]. The <sup>13</sup>C NMR spectrum of **4** had resonances for 30 C atoms including typical resonances for C3=O and C12=O at 216.9 and 212.7 ppm, respectively. The chemical shifts for C12 and C13 (50.2 ppm) in 13 $\beta$ -H ketone **4** were similar to those of 13 $\beta$ -H-12-oxo-derivatives of oleanolic acid [8] and confirmed its configuration.

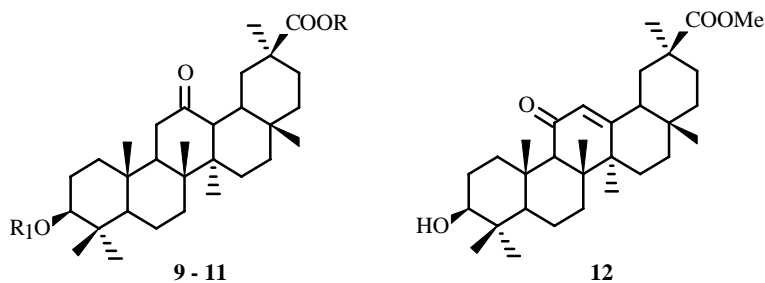
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Scheme 1

It was hypothesized that ozonolysis did not form the molar ozonide but one of canonical structures **5** or **7**, which are stabilized by elimination of oxygen. Compound **5** converts into enol **6**, which isomerizes into ketone **4**, whereas **7**, skipping the formation of epoxide **8** as proposed in the literature [5], immediately transforms into final ketone **4** because we could not isolate it among the reaction products. The 3-hydroxyl underwent ozone initiated oxidation by oxygen to an oxo group (Scheme 1).

Ozonation of 11-desoxy-GLA at -60°C in CH<sub>2</sub>Cl<sub>2</sub>:CH<sub>3</sub>OH until turning blue and reduction with Zn in CH<sub>3</sub>COOH, as described above for **4**, produced 3-hydroxyolean-12-on-30-oic acid (**9**) in 42% yield after fractional crystallization from EtOH. The <sup>13</sup>C NMR spectrum of **9** contained a resonance for carbonyl C3 at 78.7 ppm, like in the spectrum of GLA [9]. Carbonyl C12 resonated at 213.1 ppm; C13, 50.3 ppm. Molecular ions in LC/MS chemical-ionization (CI) spectra appeared at (*m/z*) [M + H]<sup>+</sup> 473 and [M - H]<sup>-</sup> 471, which agreed with the molecular formula C<sub>30</sub>H<sub>48</sub>O<sub>4</sub>. The mother liquor contained a difficultly separated mixture of **9**, GLA, and 11-deoxy-GLA (**1**), which were identified by their <sup>13</sup>C NMR spectra.



**9**: R = R<sub>1</sub> = H; **10**: R = H, R<sub>1</sub> = Ac; **11**: R = Me, R<sub>1</sub> = H

Exhaustive ozonolysis of **2** at  $-60^{\circ}\text{C}$  in  $\text{CH}_2\text{Cl}_2$  produced 12-ketone **10**, which was isolated by fractional crystallization from  $\text{CH}_3\text{OH}:\text{CH}_2\text{Cl}_2$  in 48% yield. Carbonyl C12 had CS 212.7 ppm in the  $^{13}\text{C}$  NMR spectrum of **10**; C13, 50.8 ppm. The first precipitate turned out to be the starting 3-*O*-acetate of GLA (**2**), the PMR spectrum of which had singlets for olefinic proton H12 at  $\delta$  5.30 ppm and for 3-*O*-Ac at  $\delta$  2.04 ppm.

Ozonolysis of **3** in  $\text{CH}_2\text{Cl}_2$  at  $-60^{\circ}\text{C}$  led to a mixture of oxidation products from which 12- and 11-oxo derivatives of **11** (32.5%) and **12** were isolated pure by chromatography. These were characterized by PMR and  $^{13}\text{C}$  NMR spectra and by comparing the TLC and NMR spectrum of **12** with those of a standard sample of GLA methyl ester.

The  $^{13}\text{C}$  NMR spectrum of **11** contained resonances for C3(OH) at  $\delta$  78.6 ppm and C12(=O) at 212.2 ppm. The PMR spectrum of **11** had a strong singlet for  $\text{CH}_3\text{O}$  at  $\delta$  3.72 ppm and resonances for protons of seven methyls at strong field, an  $\alpha$ -carbinol proton at  $\delta$  3.20 ppm, and  $\beta$ -H13 at  $\delta$  2.76 ppm (d,  $J = 4.1$  Hz). The  $^{13}\text{C}$  NMR spectrum of **12** was identical to that of methylglycyrrhetate [9]. Molecular ions  $[\text{M} + \text{H}]^+$  and  $[\text{M} - \text{H}]^-$  had  $m/z$  values of 487 and 485, respectively, which agreed with the formula  $\text{C}_{31}\text{H}_{50}\text{O}_4$ .

## EXPERIMENTAL

Column chromatography (CC) was performed over KSK silica gel (50-150 fraction) (ZAO Sorbopolimer) or aluminum oxide (Brockmann neutral). TLC used Sorbfil (Sorbopolimer) plates. Spots of compounds were developed with phosphotungstic acid in EtOH (20%) followed by heating at  $110$ - $120^{\circ}\text{C}$  for 2-3 min.

PMR and  $^{13}\text{C}$  NMR spectra were recorded on a Bruker AM 300 spectrometer at operating frequency 300 and 75.5 MHz in deuteropyridine or  $\text{CDCl}_3$  with TMS internal standard. Resonances in NMR spectra were assigned using COSY and HSQC correlation spectroscopy and by comparison with literature data for GLA and its derivatives [9, 10]. Molecular ions were determined by LC/MS on a Shimadzu LCMS-2010 using CI at atmospheric pressure as solutions in methanol or acetonitrile.

11-Desoxo-GLA (**1**) was prepared by reduction of GLA with Zn and HCl in dioxane as before [11], mp  $323$ - $325^{\circ}\text{C}$  (ethylacetate), lit. [11] mp  $322$ - $325^{\circ}\text{C}$ . The 3-*O*-acetate (**2**) was prepared by heating 11-desoxo-GLA in a mixture of pyridine and acetic anhydride and recrystallizing from aqueous MeOH, yield 73%, mp  $310^{\circ}\text{C}$ , lit. [12] mp  $309$ - $310^{\circ}\text{C}$ . The methyl ester of 11-desoxo-GLA (**3**) was prepared by treating a solution of **1** in MeOH with an ether solution of diazomethane as described before [10], mp  $248$ - $250^{\circ}\text{C}$  ( $\text{CHCl}_3:\text{CH}_3\text{OH}$ ), lit. [13] mp  $248$ - $249^{\circ}\text{C}$ . Solvents were purified by literature methods [14] and evaporated in vacuo at  $40$ - $50^{\circ}\text{C}$ .

**18 $\beta$ H-Olean-3,12-dion-30-oic Acid (4)**. Ozonated oxygen was passed through a solution of **1** (2 mmol, 0.94 g) in  $\text{CH}_2\text{Cl}_2$  (100-150 mL) at  $-60^{\circ}\text{C}$  until ozone appeared at the outlet of the flask (until the solution turned blue). The temperature was adjusted to  $0^{\circ}\text{C}$ . The mixture was treated with glacial  $\text{CH}_3\text{COOH}$  (20 mL) and Zn powder (2 g), stirred for 1 h, and filtered to remove Zn. The organic phase was treated with saturated  $\text{Na}_2\text{CO}_3$  solution until the pH was 7, washed with water (3  $\times$  20 mL), dried over  $\text{MgSO}_4$ , and evaporated. The dry solid (0.90 g) was chromatographed over an  $\text{Al}_2\text{O}_3$  column with elution by benzene. Fractions that were homogeneous by TLC were combined to afford **1** (0.25 g), which was identified by TLC with a marker, and crude **2** (0.34 g, 36%), which was recrystallized from EtOH,  $R_f$  0.26 ( $\text{CHCl}_3:\text{EtOH}$ , 20:1), mp  $215$ - $217^{\circ}\text{C}$ .

PMR spectrum ( $\text{CDCl}_3$ ,  $\delta$ , ppm): 0.86 (s, 3H,  $\text{CH}_3$ -28), 0.94 (s, 3H,  $\text{CH}_3$ -23), 0.96 (s, 3H,  $\text{CH}_3$ -27), 1.00 (s, 3H,  $\text{CH}_3$ -26), 1.04 (s, 3H,  $\text{CH}_3$ -24), 1.12 (s, 3H,  $\text{CH}_3$ -24), 1.18 (s, 3H,  $\text{CH}_3$ -25), 1.20-2.00 (m, CH,  $\text{CH}_2$ ), 2.20-2.80 (m, 5H,  $\text{CH}_2$ -11,  $\text{CH}_2$ -2, H-13).

$^{13}\text{C}$  NMR spectrum ( $\text{CDCl}_3$ ,  $\delta$ , ppm): 38.4 (C1), 33.8 (C2), 216.9 (C3), 47.3 (C4), 54.8 (C5), 19.5 (C6), 33.8 (C7), 41.4 (C8), 48.9 (C9), 36.6 (C10), 38.5 (C11), 212.8 (C12), 50.3 (C13), 43.8 (C14), 28.9 (C15), 26.3 (C16), 32.0 (C17), 43.8 (C18), 32.8 (C19), 42.2 (C20), 33.8 (C21), 38.3 (C22), 21.1 (C23), 25.5 (C24), 14.8 (C25), 15.8 (C26), 25.9 (C27), 26.9 (C28), 20.8 (C29), 181.9 (C30).

LC/MS ( $m/z$ ):  $[\text{M} + \text{H}]^+ = 472$ ,  $\text{C}_{30}\text{H}_{47}\text{O}_4$ , MW 471.3. The remaining fractions contained a difficultly separated mixture of ozonolysis products.

**3 $\beta$ -Hydroxy-18 $\beta$ H-olean-12-on-30-oic Acid (9)**. Ozonated oxygen was passed through a solution of 11-desoxo-GLA (2 mmol, 0.95 g) in  $\text{CH}_2\text{Cl}_2:\text{CH}_3\text{OH}$  (5:1, v/v, 150 mL) at  $-60^{\circ}\text{C}$  until it became blue. The mixture was treated with Zn powder in  $\text{CH}_3\text{COOH}$  as described above. The solid (0.88 g) was fractionally crystallized from EtOH to afford **9** (0.35 g, 42%, first precipitate),  $R_f$  0.5 ( $\text{CHCl}_3:\text{CH}_3\text{OH}$ , 5:1),  $[\alpha]_D^{20} +30^{\circ}$  ( $c$  0.1,  $\text{CH}_2\text{Cl}_2$ ).

PMR spectrum (CDCl<sub>3</sub>, δ, ppm): 0.80 (s, 3H, CH<sub>3</sub>-28), 0.88 (s, 6H, CH<sub>3</sub>-23, CH<sub>3</sub>-24), 0.94 (s, 3H, CH<sub>3</sub>-27), 1.00 (s, 3H, CH<sub>3</sub>-25), 1.15 (s, 3H, CH<sub>3</sub>-26), 1.19 (s, 3H, CH<sub>3</sub>-29), 1.30-2.40 (m, CH, CH<sub>2</sub>), 3.24 (m, 1H, α-H-3).

<sup>13</sup>C NMR spectrum (CDCl<sub>3</sub>, δ, ppm): 38.8 (C1), 26.9 (C2), 78.7 (C3), 38.5 (C4), 55.4 (C5), 16.1 (C6), 33.8 (C7), 41.6 (C8), 49.7 (C9), 37.0 (C10), 39.1 (C11), 213.1 (C12), 50.3 (C13), 43.9 (C14), 28.9 (C15), 26.5 (C16), 31.2 (C17), 45.4 (C18), 32.8 (C19), 42.2 (C20), 33.9 (C21), 38.0 (C22), 18.3 (C23), 23.4 (C24), 15.2 (C25), 15.5 (C26), 26.0 (C27), 26.5 (C28), 20.9 (C29), 181.4 (C30).

LC/MS (*m/z*): [M + H]<sup>+</sup> = 473, [M - H]<sup>-</sup> = 471, C<sub>30</sub>H<sub>48</sub>O<sub>4</sub>, MW 472.7.

The mother liquor contained a difficultly separated mixture of **9**, GLA, and **1**, which were identified by their <sup>13</sup>C NMR spectra (CDCl<sub>3</sub>): δ 184.1, 181.7, 180.8, 180.0 (3C30), 212.6 (C12=O), and 50.2 (C13) ppm (**9**); 200.1 (C11=O), 128.4 (C12), and 169.2 (C13) ppm (GLA); 144.2 (C13) and 122.5 (C12) (**1**).

**18βH-Olean-12-on-30-oic Acid 3-O-Acetate (10)**. Ozonated oxygen was passed through a solution of **2** (1 mmol, 0.48 g) in CH<sub>2</sub>Cl<sub>2</sub> (50 mL) at -60°C until it became blue. The mixture was treated as described above [Zn (1 g)/CH<sub>3</sub>COOH (20 mL)] to afford a product (0.50 g) that was fractionally crystallized from CH<sub>3</sub>OH:CH<sub>2</sub>Cl<sub>2</sub> to afford **10** (0.24 g, 48%, second precipitate), *R<sub>f</sub>* 0.33 (toluene:ethylacetate, 3:1), mp 263-265°C.

PMR spectrum (CDCl<sub>3</sub>, δ, ppm, J/Hz): 0.85, 0.88 (s, 9H, CH<sub>3</sub>-28, CH<sub>3</sub>-23, CH<sub>3</sub>-24), 0.95 (s, 3H, CH<sub>3</sub>-27), 1.14 (s, 3H, CH<sub>3</sub>-25), 1.18 (s, 3H, CH<sub>3</sub>-28), 1.20-1.70 (m, CH, CH<sub>2</sub>), 1.84, 1.86 (both s, 2H, CH<sub>2</sub>-2), 2.04 (s, 3H, CH<sub>3</sub>CO), 2.15-2.25 (m, 2H, CH<sub>2</sub>-11), 2.80 (H-13), 4.50 (dd, 1H, α-H-3, *J*<sub>1</sub> = 10.7, *J*<sub>2</sub> = 5.5).

<sup>13</sup>C NMR spectrum (CDCl<sub>3</sub>, δ, ppm): 38.4 (C1), 23.4 (C2), 80.9 (C3), 37.7 (C4), 55.2 (C5), 16.6 (C6), 33.8 (C7), 42.7 (C8), 49.5 (C9), 36.9 (C10), 38.8 (C11), 212.7 (C12), 50.2 (C13), 44.3 (C14), 29.2 (C15), 26.4 (C16), 31.9 (C17), 45.4 (C18), 32.1 (C19), 41.5 (C20), 31.1 (C21), 38.0 (C22), 18.2 (C23), 25.9 (C24), 15.3 (C25), 16.3 (C26), 25.5 (C27), 26.9 (C28), 20.8 (C29), 181.9 (C30), 21.2, 170.9 (CH<sub>3</sub>CO).

LC/MS (*m/z*): [M + H]<sup>+</sup> = 515, C<sub>32</sub>H<sub>50</sub>O<sub>5</sub>, MW 514.7.

The first precipitate (0.2 g) turned out to be starting **9**, which was identified by TLC with a marker, mp 309-310°C, and PMR spectrum. PMR spectrum (CDCl<sub>3</sub>, δ, ppm, J/Hz): 0.80, 0.85, 0.90, 1.10, 1.17 (all s, 21H, 7 CH<sub>3</sub>), 2.04 (s, 3H, CH<sub>3</sub>CO), 4.5 (dd, 1H, α-H-3, *J*<sub>1</sub> = 11.2, *J*<sub>2</sub> = 6.6), 5.30 (s, 1H, H-12).

**Ozonolysis of 11-Desoxo-GLA Methyl Ester**. Ozonated oxygen was passed through a solution of **3** (2 mmol, 0.98 g) in CH<sub>2</sub>Cl<sub>2</sub>:CH<sub>3</sub>OH until a stable blue color developed. The mixture was reduced with Zn powder in CH<sub>3</sub>COOH (2 g/20 mL) at 20-22°C for 12 h, filtered, washed with saturated Na<sub>2</sub>CO<sub>3</sub> solution and water, and dried over MgSO<sub>4</sub>. Solvent was removed in vacuo. The solid (0.98 g) was chromatographed over a SG column with elution by CHCl<sub>3</sub>:CH<sub>3</sub>OH (200:1 and 100:1, v/v) to afford a mixture (0.46 g) of **12** and **11** in a 2:1 ratio according to PMR. The mixture was separated by CC over Al<sub>2</sub>O<sub>3</sub> with elution by benzene:ethanol (100:1 and 50:1, v/v). Yield of **11**, 0.32 g (32.5%); of **12**, 0.15 g (15%). Compound **12** was identified by TLC, PMR and <sup>13</sup>C NMR spectra, and physical chemical constants compared with standard methylglycyrhetate, *R<sub>f</sub>* 0.42 (benzene:ethanol, 5:1), mp 252-254°C (EtOH), lit. [15] mp 252-256°C.

The remaining fractions contained a difficultly separated mixture of ozonation products.

**18β-Olean-12-on-3-yl-30-oic Acid Methyl Ester (11)**. *R<sub>f</sub>* 0.58 (benzene:ethanol, 5:1), mp 258-260°C (EtOH), [α]<sub>D</sub><sup>20</sup> +23° (*c* 0.06, CHCl<sub>3</sub>).

PMR spectrum (CDCl<sub>3</sub>, δ, ppm, J/Hz): 0.80 (s, 3H, CH<sub>3</sub>-28), 0.86 (s, 3H, CH<sub>3</sub>-23), 0.88 (s, 3H, CH<sub>3</sub>-24), 0.94 (s, 3H, CH<sub>3</sub>-27), 1.00 (s, 3H, CH<sub>3</sub>-25), 1.14 (s, 6H, CH<sub>3</sub>-26, CH<sub>3</sub>-29), 1.20-1.70 (m, CH, CH<sub>2</sub>), 1.75-2.00, 2.10-2.30 (m, 4H, CH<sub>2</sub>-11, CH<sub>2</sub>-2, H-9, H-18), 2.76 (d, H-13, *J* = 4.1), 3.19 (dd, 1H, α-H-3), 3.72 (s, 3H, CH<sub>3</sub>O).

<sup>13</sup>C NMR spectrum (CDCl<sub>3</sub>, δ, ppm): 38.4 (C1), 27.9 (C2), 78.6 (C3), 38.6 (C4), 55.1 (C5), 16.1 (C6), 34.2 (C7), 37.9 (C8), 49.6 (C9), 36.9 (C10), 38.8 (C11), 212.2 (C12), 50.2 (C13), 41.5 (C14), 28.7 (C15), 26.4 (C16), 31.8 (C17), 44.1 (C18), 31.3 (C19), 42.1 (C20), 32.1 (C21), 38.0 (C22), 18.2 (C23), 25.9 (C24), 15.2 (C25), 15.3 (C26), 20.9 (C27), 27.0 (C28), 26.9 (C29), 177.5 (C30), 51.5 (C31).

LC/MS (*m/z*): [M + H]<sup>+</sup> = 487, [M - H]<sup>-</sup> = 485, C<sub>31</sub>H<sub>50</sub>O<sub>4</sub>, MW 486.7.

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