Further Bisabolenes and Dammarane Triterpenes of *Commiphora kua* Resin

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From the resins of *Commiphora kua* a novel bisabolene; 6-hydroxy-2-methyl-5-(5'-hydroxy-1'(R),5'-dimethylhex-3'-enyl)-phenol together with two new dammarane triterpenes, 3 β ,16 β ,20(S),25-tetrahydroxydammar-23-ene and 3 β -acetoxy-16 β ,20(S),25-trihydroxydammar-23-ene, have been isolated. In addition, being reported are known compounds identified as 2-methyl-5-(4'(S)-hydroxy-1'(R),5'-dimethylhex-5'-enyl)-phenol, 2-acetoxyfuranodienone, 2-methoxyfuranodienone, 3 β ,16 β ,20(R)-trihydroxydammar-24-ene and its acetate derivative, 3 β -acetoxy-16 β ,20(R)-dihydroxydammar-24-ene, and β -amyrin and its acetate derivative. 2-Methyl-5-(4'(S)-hydroxy-1'(R),5'-dimethylhex-5'-enyl)-phenol displayed fungicidal activity against *Cladosporium cucumerinum* on TLC assay.

Key words aromatic sesquiterpene; dammarane triterpene; *Commiphora kua*; Burseraceae

The plant species *Commiphora kua* (Burseraceae) is known for an oleoresin exudate which is an important article of commerce.¹⁾ The plant is also used in herbal medicine as a remedy for snakebite, gonorrhoea, stomach disorders, and livestock related diseases.²⁾

Previous phytochemical studies on the plant resin afforded cycloartane triterpenes,³⁾ bisabolenes, and furanosesquiterpenoids based on a germacrane skeleton,⁴⁾ dammarane triterpenes, and octanordammaranes.⁵⁾ In continuing with our study, we now report the isolation and structural elucidation of bisabolene; 6-hydroxy-2-methyl-5-(5'-hydroxy-1'(R),5'-dimethylhex-3'-enyl)-phenol (**1**) along with two novel dammarane triterpenes; 3 β ,16 β ,20(S),25-tetrahydroxydammar-23-ene (**3**) and its acetate derivative, 3 β -acetoxy-16 β ,20(S),25-trihydroxydammar-23-ene (**4**). Also being reported from the plant are known compounds characterised as 2-methyl-5-(4'(S)-hydroxy-1'(R),5'-dimethylhex-5'-enyl)-phenol (**2**),⁶⁾ 2-methoxyfuranodienone and 2-acetoxyfuranodienone,⁷⁾ β -amyrin and its acetate derivative,⁸⁾ 3 β ,16 β ,20(R)-trihydroxydammar-24-ene (**5**) and its acetate, 3 β -acetoxy-16 β ,20(R)-dihydroxydammar-24-ene (**6**).⁹⁾ Their structures have been established based on extensive spectroscopic (UV, IR, ¹H- and ¹³C-NMR and MS) and chemical studies.

Compound **2** inhibited the growth of the plant pathogenic fungus *Cladosporium cucumerinum* on thin layer chromatography plates.

Results and Discussion

Compound **1** displayed a molecular ion peak at *m/z* 250 in the mass spectrum, corresponding to C₁₅H₂₂O₃. In its ¹H-NMR spectrum the main features of a tetra-substituted benzene ring were evident: two low field protons appearing as doublets at δ_H 6.90 and 6.70, a benzenoid methyl singlet (δ_H 2.23), a benzylic proton (δ_H 2.60), and two benzenoid hydroxyls (δ_H 7.20, D₂O exchangeable) were present. The two dimensional total correlation spectroscopy (2D TOCSY) experiments^{10,11)} together with ¹H-¹H correlation spectroscopy (COSY) correlations were used to identify the spin system for the two protons, thus locating them at positions C-3 and C-4, respectively, a fact further corroborated by nuclear

Overhauser effect spectroscopy (NOESY) cross peaks (Fig. 1). The structure of the 5'-hydroxy-1'(R),5'-dimethylhex-3'-enyl side chain at C-5 was confirmed by ¹H- and ¹³C-NMR, ¹H-detected heteronuclear multiple quantum coherence (HMQC) and heteronuclear multiple bond connectivity (HMBC) experiments.¹²⁾

Also characteristic of the ¹H-NMR spectrum were the presence of a hydroxy isopropyl moiety at δ_H 1.30 and a two-proton multiplet at δ_H 5.56. The latter resolved in benzene-*d*₆ indicating the presence of a trans double bond.⁶⁾ The ¹³C-NMR data (Table 1) ascertained these molecular features and peaks at δ_C 72.6 (C-5'), 124.7 (C-3'), and 138.2 (C-4') were in accordance with the foregoing evidence. Furthermore, the two carbons at δ_C 124.7 and 138.2 represented an internal double bond and were linked to protons at δ_H 5.50 and 5.46, respectively. The HMBC correlations (Fig. 1) confirmed the double bond to be between C-3' and C-4' and both the olefinic proton peaks correlated with δ_C 72.6 (C-5').

The configuration at C-1' was established as *R* from the cd spectrum which exhibited a positive Cotton effect at 320 nm

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Fig. 1. Pertinent Correlations in the HMBC and NOESY Spectra for Compounds 1, 3 and 4

($\Delta\epsilon$ 3.6). This was further supported by comparison of the ^1H -NMR data with those reported on similar curcumen derivatives.^{13,14} On the basis of the spectroscopic evidence accrued, the structure of compound 1 was established as 6-hydroxy-2-methyl-5-(5'-hydroxy-1'(R),5'-dimethylhex-3'-enyl)-phenol.

Compound 2, a yellow oil, showed a molecular ion peak at m/z 234 corresponding to $\text{C}_{15}\text{H}_{22}\text{O}_2$. Unlike compound 1 described above, its ^1H - and ^{13}C -NMR spectral data suggested a trisubstituted benzene ring with a monounsaturated side chain at C-5.¹⁵ In its mass spectrum, a characteristic McLafferty process involving the elimination of the side chain produced an ion at m/z 148 $[\text{M}-\text{C}_5\text{H}_{10}\text{O}]^+$ and another one at m/z 135 $[\text{M}-\text{C}_6\text{H}_{10}\text{O}]^+$, both fragments were consistent with the presence of a secondary hydroxyl function.¹⁶

The complete ^1H - and ^{13}C -NMR chemical shift assignments for 2 were deduced from concerted application of ^1H - ^1H COSY and ^1H detected 'one bond' and long range (C, H) correlation experiments as well as comparison with already reported data on the compound.⁶ In a ^1H - ^1H homonuclear chemical shift correlation spectrum, the allylic carbinol proton at δ_{H} 4.20 showed connectivities with two other protons, thus limiting the position of the hydroxyl group to C-4'. The one bond proton-carbon chemical shift correlation was established using the HMQC sequence while the assignment of quaternary carbons was obtained from the analysis of long range correlation responses over three bonds using an HMBC technique. The absolute configuration at C-4' was established as *S* by the GC modification of the Horeau method,¹⁷ which afforded an excess product of (*R*)- α -phenylbutyric acid (65% optical yield) as identified by GC and GC-MS analysis. This was further supported by the cd spectrum positive Cotton effect at 246 nm ($\Delta\epsilon$ 2.6) and the

Table 1. ^{13}C -NMR of Compounds 1–5

Carbon	1	2	3	4	5
1	142.0	154.2	38.2	39.1	38.6
2	122.0	121.0	27.4	30.5	34.5
3	123.5	130.4	75.0	76.4	73.4
4	118.3	118.8	40.0	39.6	40.1
5	131.4	146.8	55.3	56.0	55.5
6	141.4	114.0	19.5	19.2	20.3
CH ₃ -2	18.7	20.4			
1'	39.3	40.0			
2'	37.5	36.0			
3'	124.7	32.9			
4'	138.2	77.2			
5'	72.6	147.3			
6'	29.4	112.4			
Me-1'	22.0	21.8			
Me-5'	30.1	17.0			
OAc-4'					
Me-Ac					
7			35.3	34.7	35.0
8			41.2	40.6	40.3
9			51.3	50.5	50.4
10			36.5	37.0	37.0
11			22.0	21.6	22.0
12			27.1	26.8	27.0
13			51.1	49.0	51.6
14			52.0	50.3	48.2
15			43.2	41.0	44.0
16			74.6	74.4	74.0
17			42.0	41.7	39.4
18			18.3	17.7	18.3
19			16.0	15.8	16.3
20			76.3	77.30	76.0
21			25.9	26.3	26.0
22			34.4	35.2	43.5
23			127.5	127.6	22.7
24			138.4	140.3	124.6
25			81.6	81.3	131.6
26			25.3	26.3	25.8
27			25.0	24.8	18.3
28			19.0	18.3	18.9
29			22.2	21.5	19.7
30			14.0	15.2	15.8
OAc				170.4	
Me-Ac				21.0	

^1H -NMR coupling constant $J_{3',4'} = 6.8, 4.4 \text{ Hz}$.¹³)

From these spectral data, the structure of 2 was deduced to be 2-methyl-5-(4'(S)-hydroxy-1'(R),5'-dimethylhex-5'-enyl)-phenol.

The other component (3) considered novel afforded a molecular ion peak in the MS at m/z 476, calculated to be $\text{C}_{30}\text{H}_{52}\text{O}_4$. From a preliminary observation, its mass spectrum exhibited the main features of a dammarane triterpene⁹) as evidenced by fragment peaks at m/z 376, 315, 208, and 207. The ^{13}C -NMR spectrum showed a total of 30 carbons and their multiplicity assignments using DEPT established 8 methine, 8 methylene, and 8 methyl groups, and 6 quaternary carbon atoms were identified from the difference of the broad band. The HMQC, HMBC, and NOESY experiments were employed in assigning all the ^1H and ^{13}C values and by comparison with the respective spectral data for compound 5,⁹) it differed from the latter only in the side chain composition. According to the ^1H - and ^{13}C -NMR data, the side chain of 3 consisted of two OH groups on substituted quaternary carbons (C-20, δ_{C} 76.3 and C-25, δ_{C} 81.6), two germinal

methyls (C-26 and C-27, δ_{H} 1.28, with respective δ_{C} 25.3 and 25.0), and a double bond (C-23 and C-24, δ_{H} 5.40 and 5.50, δ_{C} 127.5 and 138.4). The structure of the side chain was confirmed from HMBC correlations (Fig. 1) which showed cross peaks between the proton signals of H-17 (δ_{H} 2.20), and H-22 (δ_{H} 2.40) with the carbon signal of C-20 (δ_{C} 76.3). Furthermore, the H-24 signal (δ_{H} 5.50) exhibited long range coupling with carbon signals of C-22 (δ_{C} 34.4), C-23 (δ_{C} 127.5), C-26 (δ_{C} 25.3), and C-27 (δ_{C} 25.0).

The stereochemistry of the chiral carbon C-20 sustaining OH group in equatorial orientation was defined on the basis of the cd spectrum in MeOH results which gave a positive Cotton effect at 276 nm ($\Delta\epsilon$ 2.2) that closely resembled that of guggulsterol (274 nm, $\Delta\epsilon$ 2.6) previously isolated from Indian *Commiphora mukul*.¹⁸ Thus, compound **3** was deduced to be 3 β ,16 β ,20(*S*),25-tetrahydroxydammar-23-ene.

The last compound (**4**) showed eight tertiary methyl groups at δ 1.33, 1.20, 1.06, 1.03, 0.98, 0.90, and 0.88 in the ¹H-NMR spectrum. The ¹³C-NMR exhibited 32 carbon resonances. Distortionless enhancement by polarization transfer (DEPT) experiments suggested the presence of eight methylenes, eight methines, nine methyls, and 7 quaternary carbon atoms were identified from the difference of the broad band. The HMQC, HMBC, and NOESY experiments were similarly employed in assigning all the ¹H and ¹³C values and by comparison with the respective data for **3**, compound **4** differed from the former only in the acetate peak. The stereochemistry at C-20 with an OH group in an equatorial orientation was similarly determined to be *S* using cd spectrum positive Cotton effect at 278 nm ($\Delta\epsilon$ 1.8), which was similar to that of compound **3**. These findings confirmed that **4** is an acetate derivative of **3** and therefore was identified as 3 β -acetoxy-16 β ,20(*S*),25-trihydroxydammar-23-ene.

Experimental

General Melting points (uncorrected) were determined on a Buchi 535 melting point apparatus. IR spectra were run as KBr disc on an infrared IMR-25 spectrophotometer. The electron impact (EI)-MS were measured on a 70 eV MAT 8200 A Varian MAT Bremen instrument. ¹H- and ¹³C-NMR spectra were recorded on a Bruker WM NMR spectrometer operating at 400 and 100 MHz, respectively, and were recorded in CDCl₃ using tetramethylsilane (TMS) as an internal standard. $[\alpha]_{\text{D}}$ values were obtained on a JASCO DIP-360 instrument. Capillary GC was performed on a Carlo-Erba Mega instrument, 25 m $\times$ 0.33 mm SE-30 column, initial temperature 70 °C (2 min), then rising at 5 °C/min to 100 °C followed by 10 °C/min to a final temperature of 250 °C; N₂ at 20 cm/s. Preparative GC was performed on a 3 m $\times$ 2 mm i.d. Carbowax 20 M packed column operated at 180 °C. GC-MS was obtained on a Hewlett-Packard 5890/5988A instrument in the EI mode (70 eV) connected to a 100E-series data processing system. The SE-30 column (12 m $\times$ 0.33 mm), was operated under the following conditions; initial temperature 60 °C (2 min), and final temperature 200 °C, rate 5 °C/min, He at 25 cm/s.

Resin Samples Samples were collected from trees in the Isiolo District on the main road to Wajir, Kenya in February 1996. Botanical samples including leaves, branches and pods were sampled and voucher specimens (NTR/KEFRI 96/2/CK) were deposited at the Kenya Forestry Research Institute Herbarium. Samples were stored in airtight bags and kept in a refrigerator until studied.

Extraction and Isolation The residue from steam distilled resin was extracted in the cold with EtOAc (1.01 $\times$ 2) and evaporated under reduced pressure to give 40 g of a dark brown gummy material. A portion of the residue (35 g) was chromatographed over a silica gel column, eluted first with *n*-hexane followed by the same solvent containing increasing amounts of CH₂Cl₂, EtOAc, and finally with MeOH. One hundred milliliter fractions of each were collected. A total of 150 fractions were sampled and their composition monitored by silica gel TLC in two systems; A (*n*-hexane–EtOAc 4 : 1, 2 : 1 and 1 : 1) and B (CH₂Cl₂–MeOH, 9 : 1). The spots were visualised

by spraying with acidified anisaldehyde reagent and heating. The corresponding eluates were combined into pools (I–IV).

Pool I (fractions 15–40, 5.5 g) upon low pressure column chromatography with *n*-hexane–EtOAc (4 : 1) followed by the same solvent system in the ratio 3 : 1 allowed the isolation of β -amyrin and its acetate derivative,⁸ 2-methoxyfuranodienone, and 2-acetoxyfuranodienone.⁷ Pool II (fractions 41–80, 4.5 g) afforded a viscous brown resin and was similarly purified using flash chromatography over a silica gel column with *n*-hexane–EtOAc (3 : 1, 3 : 2), 150 fractions of 20 ml each being collected. This procedure gave **1** (35.4 mg), **2** (65.5 mg), **4** (55.3 mg), and **6** (85 mg). Pool III (fractions 81–112, 5.5 g) using a similar technique as in pool II yielded 75 mg of **3**, a further 33.4 mg of **4**, 52.3 mg of **5**, and a further 26.6 mg of **1**.

Pool IV (fractions 115–150, 7.5 g) was rechromatographed over a 2% oxalic acid deactivated silica gel column with CH₂Cl₂–MeOH (9 : 1) to give an unidentified sugar compound (300 mg).

Identification of Compounds The structures of known compounds were identified by comparison of their physical and spectroscopic data with those of known standards.

Compound 1: Yellow oil [$\alpha]_{\text{D}}^{25}$ –35° (*c*=1.0, CH₂Cl₂). UV λ_{max} (MeOH) 274 (log ϵ 2.90) nm. CD (*c*=0.5, MeOH): 320 ($\Delta\epsilon$ 3.6) nm. IR (max (KBr) 3450, 1620, 1250, and 1080 cm^{–1}. ¹H-NMR (CDCl₃) δ_{H} : 7.20 (2H, s, OH-1 and OH-6, D₂O exchange), 6.90 (1H, d, J_{AB} =7.8 Hz, H-3), 6.70 (1H, d, J_{AB} =7.8 Hz, H-4), 5.56 (2H, m, H-3', H-4'), 2.60 (1H, tq, J =6.7, 6.7 Hz, H-1'), 2.40 (2H, m, CH₂-2'), 2.23 (3H, s, Me-2), 1.35 (3H, d, J =7 Hz, Me-1'), 1.30 (6H, s, Me₂-5'). ¹H-NMR (C₆D₆) δ_{H} : 7.02 (1H, d, J =7.5 Hz, H-3), 6.97 (2H, s, OH-1, OH-6), 6.85 (1H, d, J =7.5 Hz, H-4), 5.50 (1H, ddd, J =15.5, 6.5, 6 Hz, H-3'), 5.46 (1H, brd, J =15.5 Hz, H-4'), 2.70 (1H, tq, J =6.9, 6.9 Hz, H-1'), 2.10 (3H, s, Me-2), 1.25 (3H, d, J =6.6 Hz, Me-1'), 1.20 (6H, s, Me₂-5'). ¹³C-NMR data: see Table 1. Hreims [M]⁺ 250.2909 (Calcd for C₁₅H₂₂O₃, 250.2916). EI-MS (70 eV): *m/z* (%) 250 (6), 234 (10), 233, 217 (14), 174 (35), 152 (26), 141 (100), 137 (28), and 41 (27).

Compound 2: Yellow oil. [$\alpha]_{\text{D}}^{25}$ –13.5° (*c*=1.0, CH₂Cl₂). UV λ_{max} (MeOH) 275 (log ϵ 2.85) nm. CD (*c*=0.5, MeOH) 324 ($\Delta\epsilon$ 2.90) and 246 ($\Delta\epsilon$ 4.2) nm. IR ν_{max} (KBr) 3400, 1610, 1450, 1250, 870 cm^{–1}. ¹H-NMR (CDCl₃) δ_{H} : 7.01 (1H, d, J =10.7 Hz, H-3), 6.80 (1H, d, J =10.7 Hz, H-4), 6.40 (1H, s, H-6), 4.90 (1H, s, H-6_B'), 4.85 (1H, s, H-6_A'), 4.20 (1H, dd, J =6.8, 4.4 Hz, H-4'), 2.65 (1H, tq, J =6.6, 6.6 Hz, H-1'), 2.40 (3H, s, Me-2), 1.80 (2H, m, CH₂-2'), 1.50 (2H, m, CH₂-3'), 1.65 (3H, s, Me-5'), 1.20 (3H, d, J =6.0 Hz, Me-1'). ¹³C-NMR data: see Table 1. EI-MS (70 eV): *m/z* (%) M⁺ 234 (20), 216 (11), 215 (3), 201 (10), 174 (5), 159 (18), 148 (100), 135 (50), 115 (80).

Compound 3: Colourless needles, [$\alpha]_{\text{D}}^{25}$ +13.6° (*c*=1.0, CH₂Cl₂). CD (*c*=0.5, MeOH): 276 ($\Delta\epsilon$ 2.2) nm. IR ν_{max} (KBr) 3500, 1640 cm^{–1}. ¹H-NMR δ_{H} : 5.50 (1H, dd, J =14.5, 7.5 Hz, H-24), 5.40 (1H, ddd, J =14.5, 7.5, 7 Hz, H-23), 4.60 (1H, ddd, J =8.5, 7.6, 5.2 Hz, H-16), 3.75 (1H, dd, J =10.5, 4.4 Hz, H-3), 2.50 (1H, ddd, J =15.6, 9.7, 7.5 Hz, H-2_{ax}), 2.45 (1H, ddd, J =15.6, 8.0, 6.4 Hz, H-2_{eq}), 2.40 (1H, m, H-22), 2.20 (1H, ddd, J =12.5, 7.6, 4.2 Hz, H-17), 2.05 (3H, s, CH₃CO-), 1.83 (1H, dd, J =14.3, 7.1 Hz, H-15_{eq}), 1.28 (6H, s, Me₂-25), 1.15, 1.08, 1.01, 0.96, 0.93, 0.88 (18H, 6 $\times$ Me). ¹³C-NMR data: see Table 1. Hreims [M]⁺ 476.3026 (Calcd for C₃₀H₅₂O₄, 476.3032). EI-MS: *m/z* (%) 476 (2), 458 [M–H₂O]⁺ (5), 440 [M–2H₂O]⁺ (30), 433 (11), 376 (20), 359 (8), 315 (15), 298 (4), 255 (25), 226 (6), 208 (16), 207 (8), 109 (43).

Compound 4: Colourless needles, [$\alpha]_{\text{D}}^{25}$ +38.3° (*c*=0.5, CH₂Cl₂), mp 140–142 °C. CD (*c*=0.5, MeOH): 278 ($\Delta\epsilon$ 1.8 nm). IR ν_{max} (KBr) 3400, 1735, 1375, 1075 and 1040 cm^{–1}. ¹H-NMR δ_{H} : 5.71 (1H, m, H-24), 5.60 (1H, m, H-23), 4.55 (1H, dd, J =8.6, 4.6 Hz, H-3), 4.45 (1H, ddd, J =7.9, 7.7, 5.3 Hz, H-16), 2.45 (1H, ddt, J =15.7, 10, 7 Hz, H-2_{ax}), 2.0 (1H, ddd, J =14.8, 7.3, 5.0 Hz, H-2_{eq}), 2.60 (1H, d, J =11, 6.5 Hz, H-13), 1.83 (1H, dd, J =12.1, 7.2 Hz, H-17), 1.67 (1H, dd, J =12.8, 7 Hz, H-15_{eq}), 1.55 (1H, dd, J =13.7, 5.4 Hz, H-15_{ax}), 2.40 (2H, m, CH₂-22), 1.33 (6H, s, Me₂-25), 1.20, 1.06, 1.03, 0.98, 0.9, 0.88 (18H, s, 6 $\times$ Me). ¹³C-NMR data: see Table 1. Hreims [M]⁺ 518.3736 (Calcd. for C₃₂H₅₄O₅, 518.3818). EI-MS: *m/z* (%) 518 (2), 500 [M–H₂O]⁺ (2), 482 [M–2H₂O]⁺ (4), 457 (5), 452 (10), 419 (15), 402 (7), 357 (11), 341 (20), 249 (8), 208 (30), 192 (10), 189 (60), 109 (70), 43 (100).

Compound 5: Colourless needles, mp 210–212 °C, [$\alpha]_{\text{D}}^{25}$ –20° (*c*=0.60, CH₂Cl₂). IR ν_{max} (KBr) 3400, 1625 cm^{–1}. ¹H-NMR δ_{H} : 5.14 (1H, t, J =7 Hz, H-24), 4.45 (1H, ddd, J =8.5, 7.6, 5.3 Hz, H-16), 3.50 (1H, dd, J =10.4, 5.3 Hz, H-3), 1.28, 1.25, 1.05, 0.98, 0.88, 0.86, 0.80, 0.75 (24H, s, 8 $\times$ Me). ¹³C-NMR data: see Table 1. EI-MS M⁺ 460 (2), 442 [M–H₂O]⁺ (34), 424 [M–2H₂O]⁺ (18), 360 (11), 341 (20), 315 (16), 207 (33), 135 (65), 121 (30), 109 (57), 95 (45), 81 (42), 71 (20), 69 (100).

Assignment of C-4' Stereochemistry for Compound 2 To the compound (10 mg) dissolved in dry pyridine (1 ml) was added excess of ($\pm$)- α -phenylbutyric anhydride¹⁷⁾ and the mixture was allowed to stand at room temperature under stirring. After 24 h the reaction mixture was added to ice-H₂O, extracted with Et₂O, and dried (Na₂SO₄) and evaporated under reduced pressure. The residue obtained was subjected to GC analysis and the results showed an excess of (*R*)- α -phenylbutyric acid (*t*_R 14 min, 65% optical yield), an esterification product which was further confirmed with preparative GC and GC-MS analyses.

Fungicidal Test A suspension of *C. cucumerinum* prepared in a medium as recommended by Homan and Fuchs¹⁹⁾ was sprayed on developed thin layer chromatograms containing crude extract at varying concentrations. The plates were incubated in a moist atmosphere for 24 h at room temperature. Clear inhibition zones (white spots) on observed TLC plates confirmed the presence of an active compound in the extract. Fractionation of the crude extract afforded compound 2 as the active ingredient.

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