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BIANTHRONE *C*-GLYCOSIDES. 2[1]. THREE NEW COMPOUNDS FROM *ASPHODELUS RAMOSUS* TUBERS.[2]

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Abstract - Three further members of the new class of bianthrone *C*-glycosides extracted from *A*. *ramosus* tubers have been identified as (-)-10'-*C*-[β-D-xylopyranosyl]- and (-)-10'-*C*-[β-D-glucopyranosyl-(1 4)-β-D-glucopyranosyl]-1,1',8,8',10,10'-hexahydroxy-3,3'-dimethyl-10,7'-bianthracene-9,9'-dione, and 10'-deoxy-10-*epi*-ramosin.

Recently we have reported the isolation and the characterization of ramosin **1**, the first member of the new class of bianthrone *C*-glycosides obtained from the ether extract of *Asphodelus ramosus* L. tubers (1).

Now we report the isolation, from the same source, and the structural determination, based on chemical and spectroscopic evidence, of the three new members **2**-**4** of this class of natural compounds.

Comparison of the ^1H and ^{13}C-nmr data (Tables 1 and 2) of **2** and **3** with those of **1** revealed immediately that both these compounds were *C*-glycosides with the same bianthrone aglycone as **1** but with different glycone moiety.

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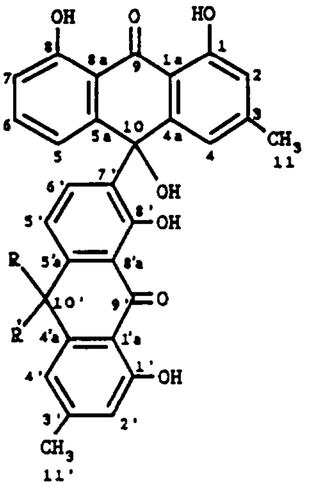
[1] Part 1 : ref. 1.

[2] This work was reported in part at the meeting "Giornate di Chimica delle Sostanze Naturali", Maggio 1989, Maratea, Italy.



1 R = OH R' = *C*-β-D-glu*p*
2 R = OH R' = *C*-β-D-xyl*p*
3 R = OH R' = β-D-glu*p*(1 4)-*C*-β-D-glu*p*
4 R = H R' = *C*-β-D-glu*p*
5a = 10-*epi*-**1**
5b = 10-*epi*-10'-*epi*-**1**
6 R, R' = O

The positive ion FAB-ms spectra of **2** and **3** showed pseudomolecular ion peaks at *m/z* 643 $(M + H)^+$ and *m/z* 857 $(M + Na)^+$, respectively. These data, together with ^{13}C-nmr chemical shifts, suggested the molecular formulae $C_{35}H_{30}O_{12}$ and $C_{42}H_{42}O_{18}$ for **2** and **3**, respectively. The occurence of the peak at *m/z* 493 $(M + H - 18 - 132)^+$ in the spectrum of **2** and of the peaks at *m/z* 655 $(M + H - 18 - 162)^+$ and 493 $(M + H - 18 - 162 - 162)^+$ in the spectrum of **3** indicated a pentose unit and an hexose disaccharide chain in structures **2** and **3**, respectively.

The identification of the sugar in **2** as a β-D-xylopyranosyl unit was inferred from the relevant $^3J_{H,H}$ values (9,2 Hz), indicating a trans-diaxial relationship among all of methine saccharide protons (Table 1).

In agreement with the disaccharide structure of the sugar chain of **3**, ^1H- and ^{13}C-nmr spectra showed both *C*-glycoside and *O*-glycoside anomeric signals at 3.26 and 83.6 ppm and at 4.15 and 102.9 ppm, respectively. The identification of monoses was achieved by acid hydrolysis in 0.1 N H_2SO_4, which yielded D-glucose and two products, one of which was identical (TLC, HPLC) with **1**. This result showed that both units of the chain were D-glucopyranose, as confirmed by the $^3J_{H,H}$ analysis of sugar protons (see Table 1).

The analysis of the ^{13}C chemical shifts, besides defining the pyranose structure for both units, allowed the identification of the carbons implied in the interglycosidic linkage, on the basis of glycosylation shift (2). Actually, both values at 78.7 or 80.0 ppm, that could be alternatively assigned to 4"-C, showed a downfield shift compared with ^{13}C data of the glycone moiety of **1** (Table 2), indicating a glycosidic linkage at this position.

Table 1. ^{1}H-nmr chemical shifts (ppm) of compounds 1-6 (apparent coupling costant in Hz) in DMSO-d_6[a]

	1	2	3	4	5a	5b	6
2	6.77 bs	6.80 bs	6.77 bs	6.76 bs	6.77 bs	6.81 bs	6.82 bs
4	6.52 bs	6.63 bs	6.51 bs	6.60 bs	6.58 bs	6.56 bs	6.65 bs
5	6.75 d (8.0)	6.75 d (7.8)	6.74 d (8.0)	6.83 d (8.0)	6.74 d (8.3)	6.76 d (7.9)	6.79 d (8.3)
6	7.49 t (8.0)	7.47 t (7.8)	7.49 t (8.0)	7.58 t (8.0)	7.50 t (8.3)	7.48 t (7.9)	7.54 t (8.3)
7	6.93 d (8.0)	6.91 d (7.8)	6.92 d (8.0)	6.96 d (8.0)	6.95 d (8.3)	6.91 d (7.9)	6.96 d (8.3)
11	2.23 s	2.28 s	2.23 s	2.21 s	2.22 s	2.25 s	2.26 s
2'	6.81 bs	6.82 bs	6.80 bs	6.71 bs	6.77 bs	6.75 bs	7.19 bs
4'	7.24 bs	7.19 bs	7.22 bs	6.89 bs	7.22 bs	7.32 bs	7.56 bs
5'	7.66 d (8.5)	7.65 d (8.3)	7.65 d (8.5)	7.31 d (8.0)	7.64 d (8.3)	7.55 d (7.9)	7.96 d (8.1)
6'	8.52 d (8.5)	8.55 d (8.3)	8.51 d (8.5)	8.47 d (8.0)	8.52 d (8.3)	8.51 d (7.9)	8.73 d (8.1)
10'				4.63 bs			
11'	2.43 s	2.43 s	2.43 s	2.37 s	2.42 s	2.40 s	2.45 s
1"	3.42 d (9.2)	3.14 d (9.2)	3.26 d (9.3)	3.26 d (9.5)	3.21 d (8.8)	3.21 d (9.3)	
2"	2.66 t (9.2)	2.93 t (9.2)	2.75 t (9.3)	3.00 t (9.5)	2.65 t (8.8)	2.75 t (9.3)	
3"	3.14 t (9.2)	3.16 t (9.2)	3.30[b,c]	3.15 t (9.5)	3.12 t (8.8)	3.13 t (9.3)	
4"	2.75 t (9.2)	2.87 m	3.00[d]	2.90 m	2.71 t (8.8)	2.55 t (9.3)	
5"a	2.86 m	3.45 dd (11.0, 5.5)	2.95[d]	2.63 m	2.86 m	2.88 m	
5"b		2.67 t (11.0, 11.0)					
6"a	3.29 dd (11.6, 5.0)		3.39[e]	3.46 dd (9.5, 5.1)	3.32 dd (11.9, 2.3)	3.43 dd (11.9, 2.3)	
6"b	3.24 dd (11.6, 3.0)		3.29[c]	3.40 dd (9.5, 4.9)	3.21 dd (11.9, 5.7)	3.03 dd (11.9, 6.6)	
1"'			4.15 d (9.6)				
2"'			2.95 t (9.6)				
3"'			3.15[f]				
4"'			3.04 t (8.1)				
5"'			3.15[f]				
6"'a			3.65 dd (13.0, 2.3)				
6"'b			3.39[e]				
Hydroxyl protons:							
1	12.24 s	12.27 s	12.24 s	12.18 s	12.22 s	12.21 s	12.22 s
8	12.31 s	12.22 s	12.24 s	12.29 s	12.30 s	12.28 s	12.30 s
10	6.88 s[g]	6.96 s[g]	6.89 s[g]	6.92 s	6.89 s[g]	6.92 s[g]	7.17 s
1'	11.59 s	11.49 s	11.59 s	11.41 s	11.57 s	11.54 s	11.60 s
8'	11.98 s	11.81 s	11.95 s	11.88 s	11.97 s	11.90 s	12.11 s
10'	6.80 s[g]	7.21 s[g]	6.83 s[g]		6.82 s[g]	6.88 s[g]	
2"	5.65 d (4.0)	6.31 d (4.0)	4.70 s[h]	5.43 d (5.9)	5.71 bs	5.60 bs	
3"	4.99 d (5.0)	5.15 d (5.5)	5.07 m[h]	5.09 d (5.2)	5.03 bs	5.02 bs	
4"	4.88 d (5.8)	4.93 d (5.5)	5.07 m[h]	4.82 d (5.2)	4.89 bs	4.88 bs	
6"	4.03 t (5.5)		4.18 t (5.0)	[i]	4.04 bs	4.00 bs	
2"'			5.29 d[h] (4.8)				
3"'			6.07 bs[h]				
6"'			4.60 t (5.5)				

[a] Assigments were obtained by comparison with 1. For compounds 2, 3 and 5b, 2D-COSY experiments were also performed. Assigments of broad singlet of 5a and 6 were confirmed through proton NOE experiments by irradiation of methyl signals. Multeplicities and coupling costants of sugar moiety protons were measured on samples treated with D_2O. Hydroxyl proton data were obtained by irradiation of H_2O resonance in saturation transfer experiments.

[b] The signal of 3"-H clearly appears as a triplet ($^3J_{2,3} = {}^3J_{3,4} = 8.7$ Hz) in a spectrum measured at 40°C.

[c,d,e,f] Overlapped signals.

[g,h] Assigments in each column may be interchanged.

[i] No signal appeared for the 6"-OH proton.

Table 2. ^{13}C-nmr chemical shifts (ppm) and DEPT of compounds 1-6 in DMSO-d_6.[a]

	1		2		3		4[b]		6	
1	161.7	C	160.9	C	160.9	C	160.8; 161.1[c]	C	161.4	C
1a	112.4	C	112.2	C	112.1	C	112.2; 112.2	C	112.1	C
2	116.9	CH	116.8	CH	116.7	CH	116.3; 116.3[d]	CH	116.5	CH
3	148.8	C	148.9	C	148.7	C	148.9; 148.9	C	147.8	C
4	120.5	CH	120.2	CH	120.1	CH	120.2; 120.2	CH	120.4	CH
4a	149.2	C	148.9	C	149.1	C	149.1; 149.1	C	149.1	C
5	119.4	CH	119.4	CH	119.2	CH	119.2; 119.2	CH	119.1	CH
5a	149.0	C	148.2	C	148.4	C	148.1; 148.1	C	148.2	C
6	137.1	CH	136.7	CH	136.8	CH	136.7; 136.7	CH	136.7	CH
7	116.4	CH	116.4	CH	116.1[c]	CH	116.0; 116.0[d]	CH	116.2	CH
8	161.4	C	161.3	C	161.4	C	161.4; 161.3	C	161.3	C
8a	114.5	C	114.3	C	114.1	C	114.9; 114.7	C	114.1	C
9	193.0[c]	C	192.7[c]	C	192.7[d]	C	193.3; 193.3[e]	C	192.5[c]	C
10	69.9	C	69.3	C	69.5	C	69.5; 69.5	C	69.5	C
11	22.0	CH_3	21.7	CH_3	21.7	CH_3	21.7; 21.7	CH_3	21.5	CH_3
1'	161.2	C	160.9	C	161.1	C	161.1; 161.1[c]	C	161.0	C
1'a	113.8	C	113.5	C	113.5	C	114.3; 114.3	C	113.7	C
2'	117.0	CH	116.2	CH	116.2[c]	CH	118.0; 119.2	CH	118.8	CH
3'	147.5	C	147.4	C	147.0	C	147.5; 146.8	C	147.7	C
4'	119.8	CH	119.2	CH	119.4	CH	121.4; 119.9	CH	123.8	CH
4'a	146.1	C	145.3	C	145.4	C	145.6; 144.6[f]	C	141.6	C
5'	116.7	CH	116.0	CH	116.3[c]	CH	115.6; 115.8[d]	CH	120.2	CH
5'a	147.0	C	147.1	C	146.8	C	142.0; 141.2[f]	C	132.4	C
6'	132.3	CH	132.2	CH	132.1	CH	132.0; 131.0	CH	132.7	CH
7'	133.9	C	133.8	C	133.6	C	132.9; 132.6	C	133.0	C
8'	157.1	C	156.4	C	156.7	C	157.2; 157.1	C	157.6	C
8'a	115.5	C	115.3	C	115.3	C	117.1; 116.7	C	115.4	C
9'	192.6[c]	C	192.2[c]	C	192.2[d]	C	192.7; 192.7[e]	C	191.7[c]	C
10'	74.9	C	75.1	C	74.8	C	44.0; 43.8	CH	181.1	C
11'	22.2	CH_3	22.0	CH_3	21.9	CH_3	21.7; 21.7	CH_3	21.4	CH_3
1"	84.7	CH	84.2	CH	83.6	CH	85.3; 84.8	CH		
2"	72.0	CH	71.4	CH	71.5	CH	70.7; 70.4	CH		
3"	78.2	CH	77.8	CH	75.9[e]	CH	78.4; 77.9	CH		
4"	70.1	CH	69.1	CH	78.7[f]	CH	70.2; 70.2	CH		
5"	81.1.	CH	69.8	CH_2	80.0[f]	CH	80.9; 80.2	CH		
6"	61.3	CH_2			60.3	CH_2	61.8; 61.1	CH_2		
1"'					102.9	CH				
2"'					73.1	CH				
3"'					76.4[e]	CH				
4"'					69.9	CH				
5"'					76.7[e]	CH				
6"'					60.9	CH_2				

[a] Assigments were obtained by comparison with 1 and, in the case of compouds 2 and 3, by 2D heteronuclear shift correlated experiments.

[b] See text.

[c,d,e,f] Assigments in each column may be interchanged.

The *β*-configuration for both anomeric carbons was deduced from the large values of $^3J_{H,H}$ of the anomeric protons at *δ* 3.26 (9.3 Hz) and at *δ* 4.15 (9.6 Hz) (Table 1).

The general appearance of ^{1}H-nmr of **4** (Table 1) suggested for this compounds a *C*-glycoside structure quite similar to that of **1**, the main differences between the ^{1}H-nmr spectra of these compounds being the lack of an alcoholic hydroxyl singlet signal and the occurence of a broad singlet at 4.63 ppm in the spectrum of **4**. In addition, the positive FAB-ms of **4** displayed pseudomolecolar ions at *m/z* 657 $(M + H)^+$ and 679 $(M + Na)^+$, which suggested the molecolar formula $C_{36}H_{32}O_{16}$. A signal at *m/z* 477 $(M + H - 18 - 162)^+$ indicated the loss of an hexose unit.

These features suggested the presence, in the structure of **4**, of an aliphatic methine proton, whose location at 10'- rather than 10-position was inferred from its chemical shift at *δ* 4.63 which fit very well for the chemical shift of a dibenzylic (3) rather than a triphenylmethylic (4) proton in an anthrone structure. The irradiation of this proton caused sharpening of the sugar signal at *δ* 3.26 (1"-H), indicating that at the 10'-position was linked the sugar moiety, for which a structure of a *β*-D-glucopyranose was suggested on the basis of the $^3J_{H,H}$ values of 1", 2" and 3" protons (Table 1).

As for the ^{13}C-nmr spectrum of **4**, this appeared more complex than expected owing to the fast (5) epimerization of the 10' center, occurred during the measure of the spectrum in DMSO-d_6.

Accordingly, in the ^{1}H-nmr spectrum which was measured before ^{13}C spectrum, only one signal for the 10'-H was displayed at *δ* 4.63, while in the ^{1}H spectrum which was measured after the ^{13}C spectrum an additional broad singlet appeared at *δ* 4.57 together with other signals in the aromatic zone. Therefore the measured ^{13}C spectrum was actually that of the 10'-epimeric mixture. In Table 2 a pair of ^{13}C chemical shifts are reported for each carbon, the values in each pair being interchangeable.

These ^{13}C data allowed us to confirm structure of **4**. In particular, the methine signal at *δ* 44.0/43.8, in accordance with the chemical shift value reported for the 10-carbon of rheinosides C and D (6), the singlet signal at *δ* 69.5 for a carbinolic carbon and the lack of a singlet at about 75 ppm, which occured in the ^{13}C spectra of **1-3**, confirmed that the 10- and not the 10' position was the hydroxylated site.

The *β*-configuration at the anomeric carbon and the pyranose form of glucose unit were substantiated by the close similarity of the ^{13}C data related to the glyconic moieties of **4** and **1**.

On the basis of these results the structure of **4** was defined as a 10'-deoxy derivative of **1**. However, the spontaneous oxidation of **4** revealed a configurational difference between these compounds.

In fact, upon standing in DMSO-$\underline{d}_6$ in the nmr tube for few months, **4** quantitatively gave a 4:6 mixture of the two products **5a** and **5b**, which resulted to be the 10'-epimeric hydroxy derivatives of **4** on the basis of the following evidence: (a) the FAB-mass spectrum of both compounds showed a pseudomolecular ion peak at $\underline{m}/\underline{z}$ 671 $(M - H)^-$; (b) in their ^{13}C-nmr spectra, a quaternary carbon signal at about δ 75 (see the 10' carbon of **1** in Table 1) appeared in place of the 10'-CH signal about at δ 46 of **4**; (c) no signal attributable to the 10'-H appeared in their ^{1}H-nmr spectra (Table 1).

While the chromatographic behaviour of **5b** was quite different from that of **1**, that one of **5a** did not allow to clearly establish if it was identical or diasteroisomeric with **1**. Therefore, CD curves for compounds **5a**, **5b** and **1** were measured.

The comparison of CD curves of both **5a** and **5b** with the curve of **1** (fig.1) immediately showed that none of the two artefacts was identical with **1**. Therefore both **5a** and **5b** had to be epimers of **1** at the 10-position, namely the native compound **4** was 10'-deoxy-10-$\underline{epi}$-**1**.

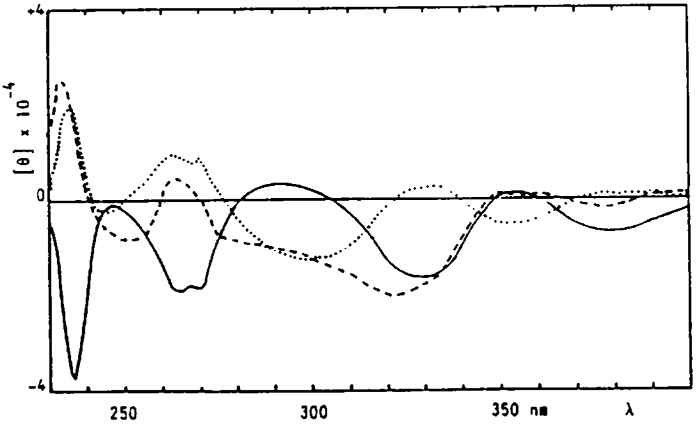


Fig. 1
CD curves of **1** (——), **5a** (---) and **5b** (...) in MeOH.

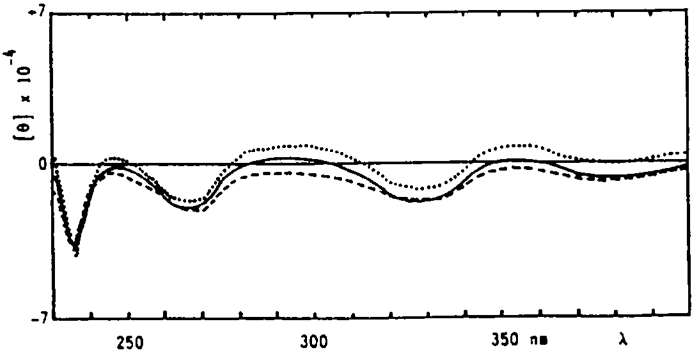


Fig. 2
CD curves of **1** (——), **2** (---) and **3** (...) in MeOH.

As for CD curves of 10'-epimers **5a** and **5b**, it was worth noticing that they showed Cotton effects of the opposite sign at longer wavelength and of the same sign at the shorter one. This might suggest that the transitions related to the longer wavelength Cotton effects were more affected by 10'- than 10-configuration, whereas the ones related to the shorter wavelength Cotton effects were more affected by 10-configuration.

Accordingly, the CD curve of **1**, which is epimer at 10-position of both **5a** and **5b**, showed Cotton effects at short wavelength with opposite sign respect to the ones displayed by **5a** and **5b**.

In the light of this result, the comparison of the CD curves of **2**, **3** and **1** (fig.2) indicated that the configurations at the 10- and at the 10'-positions were the same for all of these three compounds.

In addition to compounds **2**-**4**, isolated from the ether extract of _A. ramosus_, compound **6** was isolated from the hexane extract (see Experimental part). On the basis of its spectral properties, **6** was identified to be 10-(chrysophanol-7'-yl)-10-hydroxychrysophanol-9-anthrone (7). The ^{13}C-nmr data of **6**, not yet reported, are described in Table 2.

Work is in progress to characterize further minor components of this new class of _C_-glycosides.

EXPERIMENTAL

^{1}H-nmr (400.135) and ^{13}C-nmr (100.614) spectra were recorded in DMSO-$\underline{d}_6$ with a 400-AM FT-nmr spectrometer (Bruker), equiped with a dual probe. The DEPT and 2D proton-proton and carbon-proton shift correlated experiments were performed as reported (1). UV spectra were measured in methanol with a Cary-210 spectrometer. CD curves were measured in methanol with a JASCO J-600 dichrograph. Mass spectra were recorded with a VG ZAB-2SE instrument equiped with a FAB source. Rotations were determined in methanol solutions on a Perkin Elmer mod. 141 polarimeter. Hplc was performed with a Varian 5060 instrument using a UV detector.

Isolation of compouds **2**-**6**. The ether extract (3 g) of _Asphodelus ramosus_ tubers was submitted to DCCC separation as reported (1). Besides fractions A, B, C, D, E, and F (1), an additional more polar fraction G has been now collected.

Fraction E (80 mg) was submitted to hplc (Lichrosphere RP-18, 7:3 MeOH-H_2O, 0.7 ml/min, UV detection at 260 nm,). Two fractions were collected. Upon solvent evaporation, the shorter retention-time fraction gave compound **2** (50 mg) as a yellow amorphous solid, $[\alpha]_D^{25} = -32^o$ (c = 0.3, MeOH). ^{1}H- and ^{13}C-nmr spectra: see Tables. FAB-ms: see text. UV: λ_{max}^{MeOH} nm (ϵ): 374 (19900), 302 (15200), 268 (14400). The other fraction yielded, after evaporation, compounds **4** (20 mg) as a yellow amorphous solid. ^1H- and ^{13}C-nmr: see Tables. FAB-ms: see text.

The fraction G (45 mg) was purified by hplc (Lichrosphere RP-18, 6:4 $MeOH-H_2O$, 0.7 ml/min, UV detection at 260 nm) to give compound **3** (25 mg) as an amorphous solid, $[\alpha]_D^{25} = -60^\circ$ (c = 0.1 MeOH). ^1H- and ^{13}C-nmr spectra: see Tables. FAB-ms: see text. UV: λ_{max}^{MeOH} nm (ϵ): 377 (20200), 304 (15500), 269 (14800).

The hexane extract (1g) (1) of *Asphodelus ramosus* tubers was submitted twice to CC (silica gel; 7:3 hexane-Et_2O and 9:1 toluene-AcOEt) and then to PLC (silica-gel; 9:1 toluene-AcOEt) to give an amorphous yellow solid (3 mg), which was identified as 10-(chrysophanol-7'-yl)-10-hydroxy-chrysophanol-9-anthrone 6 (7) on the basis of its NMR data (see Table).

Acid hydrolysis of compound **3**. A sample (2 mg) of **3** was hydrolyzed with 0.1 N H_2SO_4 (0.5 ml) at 100°C for 2 hours. After neutralization with sat. $NaHCO_3$, solvent was evaporated. The methanol-soluble part of the residue showed a spot at the same Rf as **1** on TLC (silica gel, 85:15 $CHCl_3$-MeOH, 2 runs) and a peak with the same retention time as **1** at HPLC (Lichrosphere RP-18, 7:3 $MeOH-H_2O$, 0.7 ml/min, UV detection at 260 nm). The water-soluble part of the residue showed a spot at the same Rf as glucose on TLC (silica gel 14:6:1 $CHCl_3-MeOH-H_2O$).

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