Studies on the Chinese Crude Drug "Shoma." X.[1] Three New Trinor-9,19-cyclolanostanol Xylosides, Cimicifugosides H-3, H-4 and H-6, from Cimicifuga Rhizome and Transformation of Cimicifugoside H-1 into Cimicifugosides H-2, H-3 and H-4

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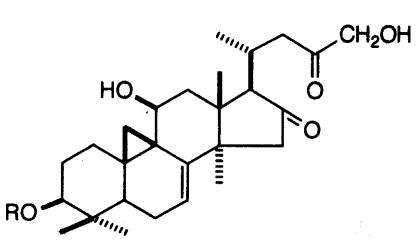
Three trinor-triterpenol glycosides were isolated from a batch of commercial Cimicifuga Rhizome: cimicifugoside H-3 (1), $C_{32}H_{48}O_9$, mp 249—251 °C, $[\alpha]_D$ −22.3°, cimicifugoside H-4 (2), $C_{32}H_{48}O_9$, mp 265—267 °C, $[\alpha]_D$ −75.0°, and cimicifugoside H-6 (3), $C_{32}H_{48}O_{10}$, mp 275—276 °C, $[\alpha]_D$ −64.3°. On the basis of chemical and spectral data, the structure of 1 was proposed to be 11β,24-dihydroxy-3β-(β-D-xylopyranosyloxy)-25,26,27-trinor-9,19-cyclolanost-7-ene-16,23-dione. Cimicifugoside H-4 (2), 11β,16α,24α-trihydroxy-3β-(β-D-xylopyranosyloxy)-25,26,27-trinor-9,19:16,24-dicyclolanost-7-en-23-one, seems to be generated from intramolecular aldol condensation between C-16 and C-24 of 1. Cimicifugoside H-6 (3) is the 15α-hydroxy derivative of 2. Cimicifugoside H-2 (5), which has already been obtained from cimicifugoside H-1 (4) under an acidic condition, was found to give 1, 2 and an α-hydroxy enone (2a) under an alkaline condition.

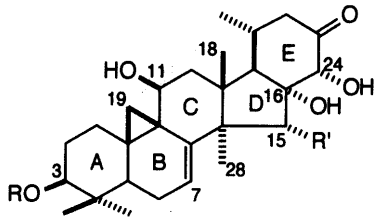
Key words cimicifugoside (H-3, H-4 and H-6); Cimicifuga Rhizome; trinor-9,19-cyclolanostanol xyloside; triterpenol glycoside; Cimicifuga triterpene reactivity

The rhizomes of the genus *Cimicifuga* (Ranunculaceae) have been used as an antipyretic and an analgesic remedy in Chinese traditional medicine. We and other groups have isolated several triterpenol glycosides, such as cimigenol xyloside,[2] acetylshengmanol xyloside,[3] 24-*O*-acetylhydroshengmanol xyloside[4] and cimicifugoside,[5] in addition to cinnamic acid derivatives,[6] chromones[7] and indolinones.[8] We recently reported the isolation and the structure determination of cimicifugosides H-1, H-2 and H-5.[1] In our continuing search, we isolated three new trinor-triterpenol glycosides, cimicifugosides H-3 (**1**), H-4 (**2**) and H-6 (**3**), from a batch of commercial Cimicifuga Rhizome. We have already briefly reported the chemical structures of cimicifugosides H-1, H-3 (**1**) and H-4 (**2**).[9] This paper presents details of the structure elucidation of **1**, **2** and **3**, and the transformation of cimicifugoside H-1 into cimicifugosides H-2, H-3 and H-4. The isolation and purification of these compounds are described in detail in the experimental section.

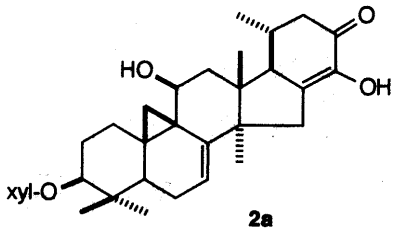
Cimicifugoside H-3 (**1**) was obtained as colorless needles, mp 249—251 °C, $[\alpha]_D$ −22.3°. Its molecular formula was determined as $C_{32}H_{48}O_9$ on the basis of the FAB-MS result. The IR spectrum of **1** showed absorptions at 1730 and 1710 cm^{-1} due to two carbonyl groups. The ^{1}H-NMR spectrum of **1** exhibited the presence of a secondary and four tertiary methyl groups (δ 1.02—1.40), a trisubstituted double bond (δ 5.13, br d, J=6 Hz), a cyclopropane methylene (δ 0.95 and 1.94, each d, J=4 Hz), two carbinyl methines (δ 3.58, dd, J=11, 4 Hz and 4.50, m), an AB type methylene bearing an oxygen atom (δ 4.48



1 ; R=β-D-xylopyranosyl
1a ; R=H



2 ; R=β-D-xylopyranosyl, R'=H
2b ; R=H, R'=H
3 ; R=β-D-xylopyranosyl, R'=OH



2a

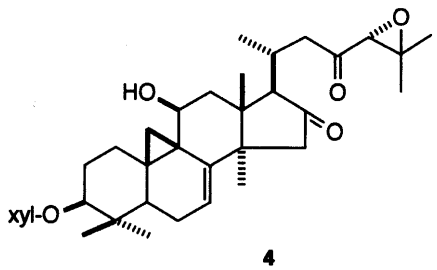


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Chart 1

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and 4.59, each d, $J=18$ Hz) and an anomeric proton (δ 4.87, d, $J=7$ Hz).

The ^{13}C-NMR spectrum of **1** was very similar to those of cimicifugosides H-1 (**4**) and H-2 (**5**),[1] except for the signals assignable to side chain C-22 through C-27. These ^{1}H- and ^{13}C-NMR data suggested that **1** is a trinor-9,19-cyclolanostanol 3-*O*-β-D-xylopyranoside with $\Delta^{7(8)}$ and a hydroxyl group at C-11, resulting from a loss of three carbons, C-25, -26 and -27, of **5**.

The ^{13}C-NMR spectrum of **1** showed 32 signals including two methines bearing an oxygen atom at δ 88.4 (C-3) and 62.9 (C-11), a carbinyl methylene at 69.2 (C-24) and two ketonic carbons at δ 218.2 (C-16) and 210.8 (C-23). In addition, the spectrum also gave information about the sugar moiety: five oxygenated carbons assignable to a β-D-xylopyranose were observed [δ 107.3 (C-1), 75.4 (C-2), 78.4 (C-3), 71.1 (C-4), 67.0 (C-5)].

The ^{1}H–^{1}H shift correlation spectroscopy (COSY) of **1** disclosed the partial structures a, b, c, d, e, f and g in Fig. 1. When the partial structures a to f were applied to a 9,19-cyclolanostanol skeleton, rings A, B, C and D of the genin part were presumable, as in the case of cimicifugosides H-1 (**4**) or H-2 (**5**) (Chart 1). On the other hand, the reducing nature of **1** was demonstrated by a positive coloration (blue) with an alkaline blue tetrazolium reagent on TLC, implying the presence of an α-ketol system in the molecule . This finding allowed us to extend the

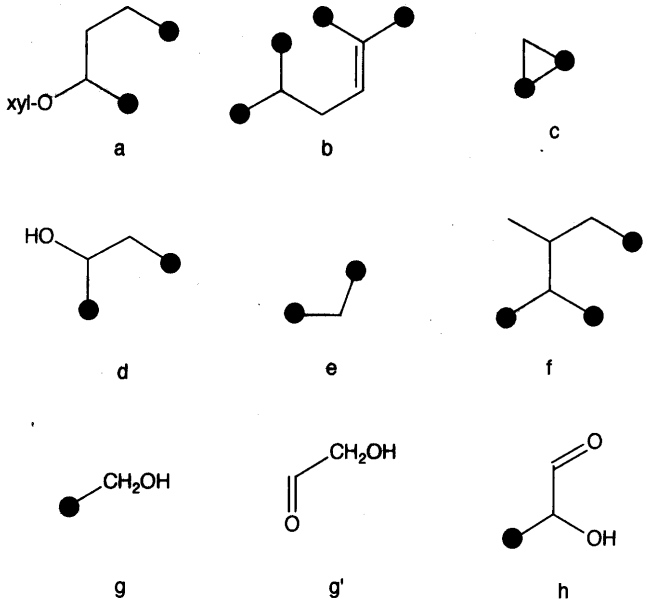

Fig. 1. Partial Structures of Compounds

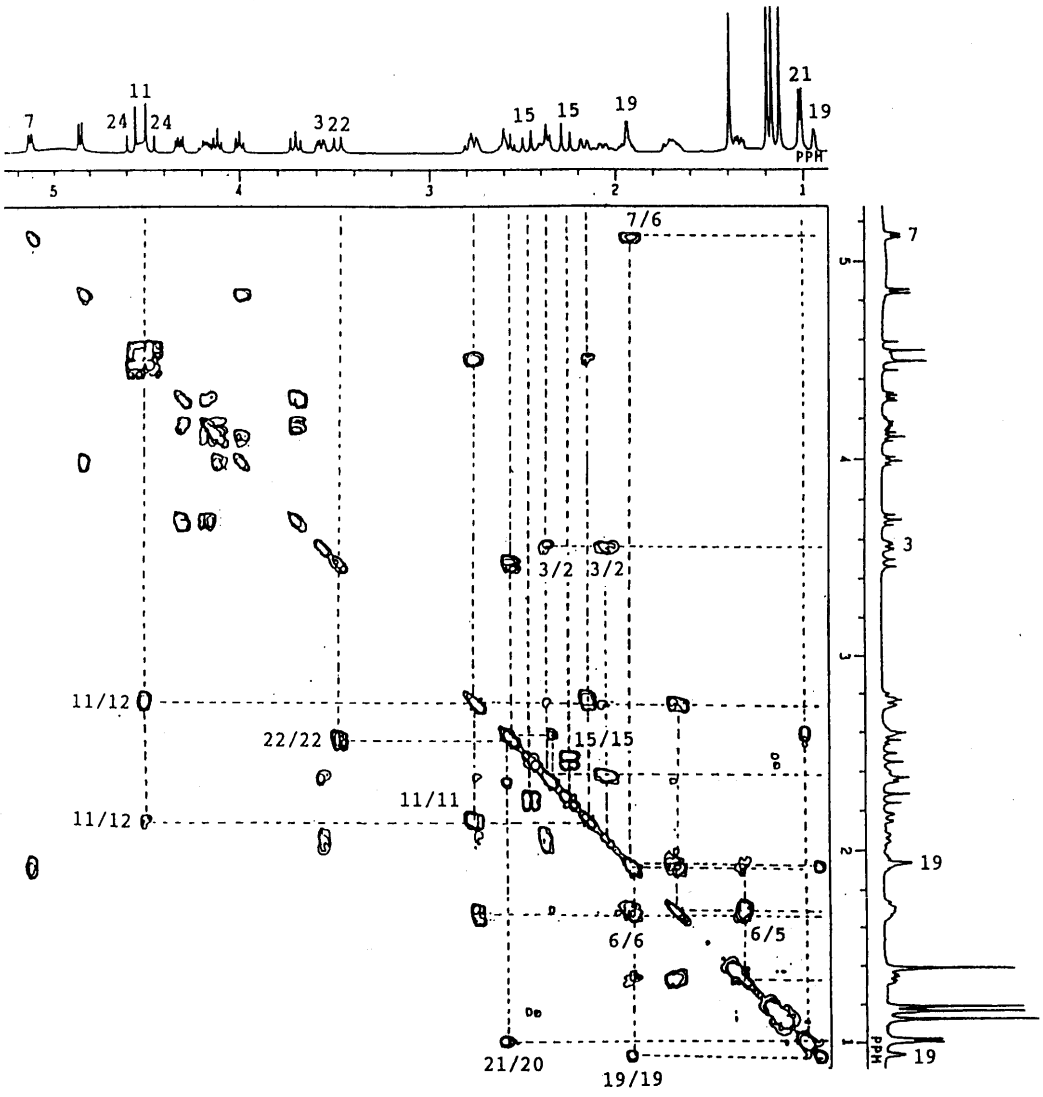

Fig. 2. ^{1}H–^{1}H COSY Spectrum of Compound **1** in Pyridine-d_5

Table 1. ^{13}C-NMR Chemical Shifts of Compounds **1**, **1a**, **2**, **2a**, **2c** and **3**

	1[a]	**1a**[a]	**2**[a]	**2a**[a]	**2c**[b]	**3**[a]
1	27.4	27.7	27.5	27.5	38.3	27.4
2	29.7	30.9	29.9	29.9	36.8	29.6
3	88.4	78.0	88.5	88.5	84.9	88.6
4	40.7	40.5	40.7	39.8	45.4	40.6
5	43.8	43.5	44.2	40.8	58.1	43.9
6	22.0	22.3	22.1	22.1	24.6	22.0
7	115.4	115.5	113.8	114.9	124.7	114.1
8	147.2	147.2	149.4	148.1	145.0	148.0
9	27.5	27.5	27.5	27.5	133.1	27.5
10	29.3	29.7	29.2	29.2	88.5	29.0
11	62.9	63.1	63.6	63.4	124.9	63.3
12	47.3	47.3	48.9	47.2	24.9	49.4
13	44.4	44.4	46.4	45.1	45.8	42.4
14	46.1	46.1	50.9	50.2	50.5	52.6
15	49.7	49.8	48.7	47.0	46.9	77.0
16	218.2	218.3	82.0	139.5	81.3	79.1
17	61.3	61.3	63.6	56.8	60.6	61.5
18	20.1	20.2	21.2	20.1	23.3	21.5
19	18.6	18.7	18.8	18.7	40.9	18.5
20	27.7	27.7	25.9	26.0	24.7	25.6
21	20.3	20.3	20.7	19.4	20.7	20.7
22	44.6	44.6	44.9	44.0	43.3	45.0
23	210.8	210.8	211.2	194.5	210.9	210.9
24	69.2	69.2	82.3	145.5	81.0	82.1
28	27.7	27.8	28.1	28.0	25.5	19.8
29	25.9	26.3	26.0	26.0	25.4	25.9
30	14.5	13.8	14.6	14.5	18.0	14.5
xyl-1	107.3		107.4	107.4		107.1
xyl-2	75.4		75.5	75.5		75.1
xyl-3	78.4		78.5	78.5		78.1
xyl-4	71.1		71.2	71.2		70.9
xyl-5	67.0		67.0	67.1		66.8

xyl-, β-D-xylopyranosyl. *a*) δ value in pyridine-d_5 and *b*) in $CDCl_3$.

partial structure g to g'. The partial structure f corresponds to C-17, -20, -21 and -22, and g', to C-23 and -24. Namely, the structure of **1** was presumed to be as shown in Chart 1.

Enzymatic hydrolysis of **1** with Cellulase T [Amano] 4 afforded a diketonic genin (**1a**), amorphous white powder, $C_{27}H_{40}O_5$, and xylose.

The ^{1}H-NMR spectrum of **1a** exhibited the presence of a cyclopropane methylene, a trisubstituted double bond, two carbinyl methines (3-H and 11-H) and a carbinyl methylene (24-H_2), indicating that **1a** was a genuine genin of **1**. On comparison of the ^{13}C-NMR spectra of **1a** and **1**, the signal due to C-3 showed a downfield shift ($\Delta\delta$ value; +10.4: glycosylation shift).[10] So, the β-D-xylopyranose was bound to C-3 of the genin (**1a**).

All signals in the ^{1}H- and ^{13}C-NMR spectra of **1** were assigned with the aid of ^{1}H–^{1}H COSY and ^{1}H–^{13}C COSY spectra (Tables 1 and 2).

From the above evidence, cimicifugoside H-3 (**1**) was determined to be 11β,24-dihydroxy-3β-(β-D-xylopyranosyloxy)-25,26,27-trinor-9,19-cyclolanost-7-ene-16,23-dione.

Another new trinor-triterpenol xyloside **2**, $C_{32}H_{48}O_9$, mp 265—267°C, $[\alpha]_D$ −75.0°, was isolated as colorless needles, and named cimicifugoside H-4. The IR spectrum of **2** showed the absorption at 1720 cm^{-1} due to a carbonyl group. In the ^{13}C- and ^{1}H-NMR spectra of **2**, the chemical shifts assignable to rings A, B and C were very similar to those of **1** (Tables 1 and 2). The differences observed in the ^{13}C-NMR spectra were in the chemical shifts ascribable to C-16 [δ_C 82.0 (s) for **2** and 218.2 (s) ppm for **1**] and C-24 [δ_C 82.3 (d) for **2** and 69.2 (t) for **1**]. In the ^{1}H-NMR spectra, some differences were also observed between **1** and **2**: the signals assignable to 22-H_2 [δ 2.40 and 2.48 for **2** and 2.60 and 3.50 ppm for **1**], 24-H [δ 4.45 (s) for **2**, and 4.48 and 4.59 ppm (each d, J = 18 Hz) for **1**] and 28-H_3 [δ 1.56 (s) for **2** and 1.17 (s) for **1**].

The ^{1}H–^{1}H COSY spectrum of **2** showed the presence of the same partial structures as those of **1**, except for the partial structure g' in Fig. 1. However, the reducing nature of **2** was also demonstrated by a positive coloration (blue) with an alkaline blue tetrazolium reagent on TLC, implying the existence of an α-ketol structure in the molecule. When the partial structures a to f, an α-ketol structure (h) and an isolated quaternary carbon bearing an oxygen atom [δ_C 82.0 (s)] were applied to the 25,26,27-trinor-9,19-cyclolanostanol skeleton, the structure **2** (Chart 1) having a new ring E formed by linkage between C-16 and C-24 was presumable.

The stereochemistry at C-16 and -24 was deduced from difference nuclear Overhauser effect (NOE) experiments. On irradiation at 18-H_3 [δ 1.24 (s) ppm], the signal intensity of 24-H [δ 4.45 (s) ppm] increased. Irradiation at 24-H enhanced the signal intensity of 18-H_3. From these NOE results for **2**, and a consideration of a Dreiding model, it was concluded that such NOE's could only arise in the case of D/E *cis*-ring junction and 24α-OH. It follows that the 16- and 24-hydroxy groups must both be in α-configuration.

On alkaline treatment with potassium hydroxide in methanol, **2** afforded an α-hydroxy enone **2a**, $C_{32}H_{46}O_8$, as a white powder. The ^{13}C-NMR spectrum of **2a** exhibited the presence of a tetrasubstituted double bond [δ 139.5 (s) and 145.5 ppm (s)]. The ^{1}H-NMR spectrum of **2a** lacked the signal due to 24-H of **2** (δ 4.45 ppm, s). Compound **2a** seemed to be a dehydration product of **2** formed through elimination of H_2O between a tertiary hydroxyl group at C-16 and a hydrogen at C-24. The signal assignable to 28-H_3 showed an upfield shift from δ 1.56 in **2** to 1.16 ppm in **2a**, owing to the anisotropic effect of the double bond at C-16(24) and the effect of the distorted E ring.[11] The signal assignable to 24-OH showed a downfield shift to δ 10.27 ppm (an enol-acidic hydroxyl) in **2a**. Therefore, **2a** seemed to have an α-hydroxy enone system on ring E. The UV spectrum of **2a** showed an absorption maximum at λ_{max} 273 nm (ε = 18800) (Calcd 279 nm),[12] strongly supporting the structure **2a** in Chart 1.

Enzymatic hydrolysis of **2** with Cellulase T [Amano] 4 in 1 N acetic acid–1 N sodium acetate buffer (pH 5) yielded a genuine aglycone (**2b**), mp 260—261 °C, $C_{27}H_{40}O_5$, and an artificial aglycone (**2c**), mp 232—233 °C, $C_{27}H_{38}O_4$, in addition to xylose. The ^{1}H-NMR spectrum of **2b** exhibited the presence of a cyclopropane methylene (δ_H 1.08 and 2.05, each d, J = 3 Hz) and a carbinyl methine at C-11 (δ 4.62, m). The ^{1}H- and ^{13}C-NMR data suggested that **2b** is the genuine aglycone of **2**, which is identical with foetidinol as judged from a comparison of the data with those in the literature.[13]

The positive ion FAB-MS of **2c** exhibited a *quasi*-molecular ion peak at m/z 427 $(M+H)^+$, which corre-

Table 2. ^{1}H-NMR Chemical Shifts of **1**, **1a**, **2**, **2a**, **2c** and **3** (Coupling Constants in Parenthesis)

	1[a]	1a[a]	2[a]
1	1.68, 2.78	1.65, 2.68	1.80, 2.75
2	2.05, 2.35	2.03 (2H)	2.00, 2.36
3	3.58 dd (11, 4)	3.57 m	3.56 dd (11, 4)
5	1.34 dd (13, 6)	1.30	1.40
6	1.70, 1.92	1.73, 1.94	1.80, 1.90
7	5.13 br d (6)	5.15 br d (6)	5.19 br d (6)
11	4.50 m	4.50 m	4.56 m
12	2.17 dd (14, 4), 2.75	2.14, 2.77	2.05, 2.79
15	2.27 d (18), 2.42 d (18)	2.23 d (18), 2.46 d (18)	2.19 d (14), 2.50 d (14)
17	2.35	2.33	2.20
18	1.20 s	1.19 s	1.24 s
19	0.95 d (4), 1.94 d (4)	0.97 d (4), 1.91 d (4)	1.01 d (4), 2.02 d (4)
20	2.61	2.56	2.20 m
21	1.02 d (6)	1.01 d (6)	0.92 d (5)
22	2.60, 3.50	2.56, 3.45 dd (11, 7)	2.40, 2.48
24	4.48 d (18), 4.59 d (18)	4.42 d (18), 4.53 d (18)	4.45 s
28	1.17 s	1.16 s	1.56 s
29	1.40 s	1.25 s	1.37 s
30	1.14 s	1.15 s	1.13 s
xyl-1	4.87 d (7)		4.83 d (7)
xyl-2	4.03		3.97 m
xyl-3	4.10		4.09 t (9)
xyl-4	4.18		4.16 m
xyl-5	3.70 dd (11, 11), 4.33 dd (11, 5)		3.68 dd (10, 10), 4.30 dd (10, 5)
24-OH			6.08

	2a[a]	2c[b]	3[a]
1	1.75, 2.82	1.53, 1.92	1.73, 2.78
2	2.10, 2.40	1.72, 1.72	2.08, 2.38
3	3.60 dd (9, 4)	3.76 d (5)	3.62 dd (12, 4)
5	1.40	1.43 br d (5)	1.43
6	1.75, 2.01	1.93, 2.25	1.78, 2.08
7	5.33 d (6)	5.47 dd (9, 6)	6.25 d (6)
11	4.56 m[c]	5.43 d (6)	4.62 m
12	2.05, 2.79	2.10, 2.40	2.03, 2.79
15	Overlapped with other signals	2.19 d (15), 2.45 d (15)	4.77 s
17	Overlapped with other signals	2.30	2.05
18	1.16 s	0.96 s	1.30 s
19	0.99 d (4), 1.97 d (4)	3.19 br d (14), 2.55 d (14)	1.07 d (4), 2.02 d (4)
20	1.98	2.30	2.13
21	0.88 d (6)	1.05 d (6)	0.92 d (6)
22	Overlapped with other signals	2.30, 2.38	2.10, 2.43
24		4.15 s	4.54 s
28	1.16 s	1.19 s	1.52 s
29	1.42 s	1.02 s	1.42 s
30	1.08 s	0.80 s	1.16 s
xyl-1	4.89 d (7)		4.89 d (7)
xyl-2	4.05 t (8)		4.03
xyl-3	4.16 t (8)		4.15
xyl-4	4.20 m		4.23
xyl-5	3.73 dd (11, 11), 4.35 dd (11, 4)		3.73 dd (11, 11), 4.34 dd (11, 5)
24-OH	10.27 br s		

xyl, β-D-xylopyranosyl. *a*) δ value in pyridine-d_5 and *b*) in $CDCl_3$. Signal assignments were based on ^{1}H–^{1}H COSY spectra. *c*) On addition of D_2O, this signal changed into dd (8, 4).

sponds to a dehydration product of **2b** under the acidic condition. The UV spectrum of **2c** showed absorption maxima at λ_{max} 243 (ε=18400), 249 (19700), 259 (sh, 14000) and 284 nm (1210).[5] The ^{1}H-NMR spectrum of **2c** exhibited the presence of a secondary and four tertiary methyl groups (δ 0.80—1.19 ppm), two oxygen-bearing methines (δ 3.76, d, J=6 Hz and 4.15 ppm, s) and two trisubstituted double bonds (δ 5.43, d, J=6 Hz and 5.45 ppm, dd, J=10, 6 Hz), but the signal due to the cyclopropane methylene was not observed.

The ^{13}C-NMR spectrum of **2c** showed signals ascribable to two methine carbons bearing an oxygen atom, two trisubstituted double bonds, two oxygen-bearing quaternary carbons and a ketonic carbon. From a consideration of the ^{1}H–^{1}H COSY spectrum (Fig. 4), six partial structures A, B, C, D, E and F, as shown in Fig. 3, were revealed.

On the basis of these findings, the structure of **2c** was

decided to be as shown in Chart 1. This transformation of **2** into **2c** under the acidic condition is shown in Chart 2. A similar reaction is known in the chemistry of *Cimicifuga* triterpenes, *i.e.*, the transformation of cimicifugoside into cimicifugenin A.[5]

From these results, cimicifugoside H-4 (**2**) was determined to be 11β,16α,24α-trihydroxy-3β-(β-D-xylopyranosyloxy)-25,26,27-trinor-9,19:16,24-dicyclolanost-7-en-23-one.

The last xyloside **3**, named cimicifugoside H-6, was obtained as colorless needles, mp 275—276 °C, $[\alpha]_D$ −64.3°. Its molecular formula was determined to be $C_{32}H_{48}O_{10}$ on the basis of the FAB-MS result. The ^{1}H-NMR spectrum of **3** exhibited the presence of a cyclopropane methylene, a trisubstituted double bond and a secondary and four tertiary methyl groups. The spectrum was very similar to that of **2**, but two different signals were observed at δ 6.25 (br d, J=6 Hz, 7-H) and 4.77 ppm (s, 15-H).

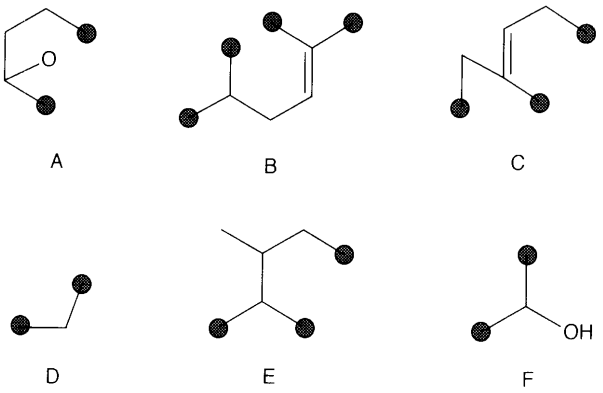

Fig. 3. Partial Structures of Compound **2c**

The ^{13}C-NMR spectrum of **3** was very similar to that of **2**, except for the signals assignable to C-15 and C-28 (Me). The spectrum showed a ketonic signal at δ 210.9 (C-23), a trisubstituted double bond at 148.0 (C-8) and 114.1 (C-7) and ten oxygenated carbon signals including those due to β-D-xylopyranose at 88.6 (C-3), 63.3 (C-11), 77.0 (C-15), 79.1 (C-16), 82.1 (C-24), 107.1 (xyl-1), 75.1 (xyl-2), 78.1 (xyl-3), 70.9 (xyl-4) and 66.8 (xyl-5).

The ^{1}H–^{1}H COSY spectrum of **3** showed the same partial structures as those of **2**, except for partial structure e in Fig. 1. The partial structures **a**, **b**, **c**, **d**, **f** and **h** and an isolated methine corresponding to an NMR signal bearing an oxygen atom [δ_C 77.0 (d) and δ_H 4.77 (br s)] were placed to form structure **3** in Chart 1. Thus, cimicifugoside H-6 (**3**) was considered to be the 15-hydroxylated derivative of cimicifugoside H-4 (**2**).

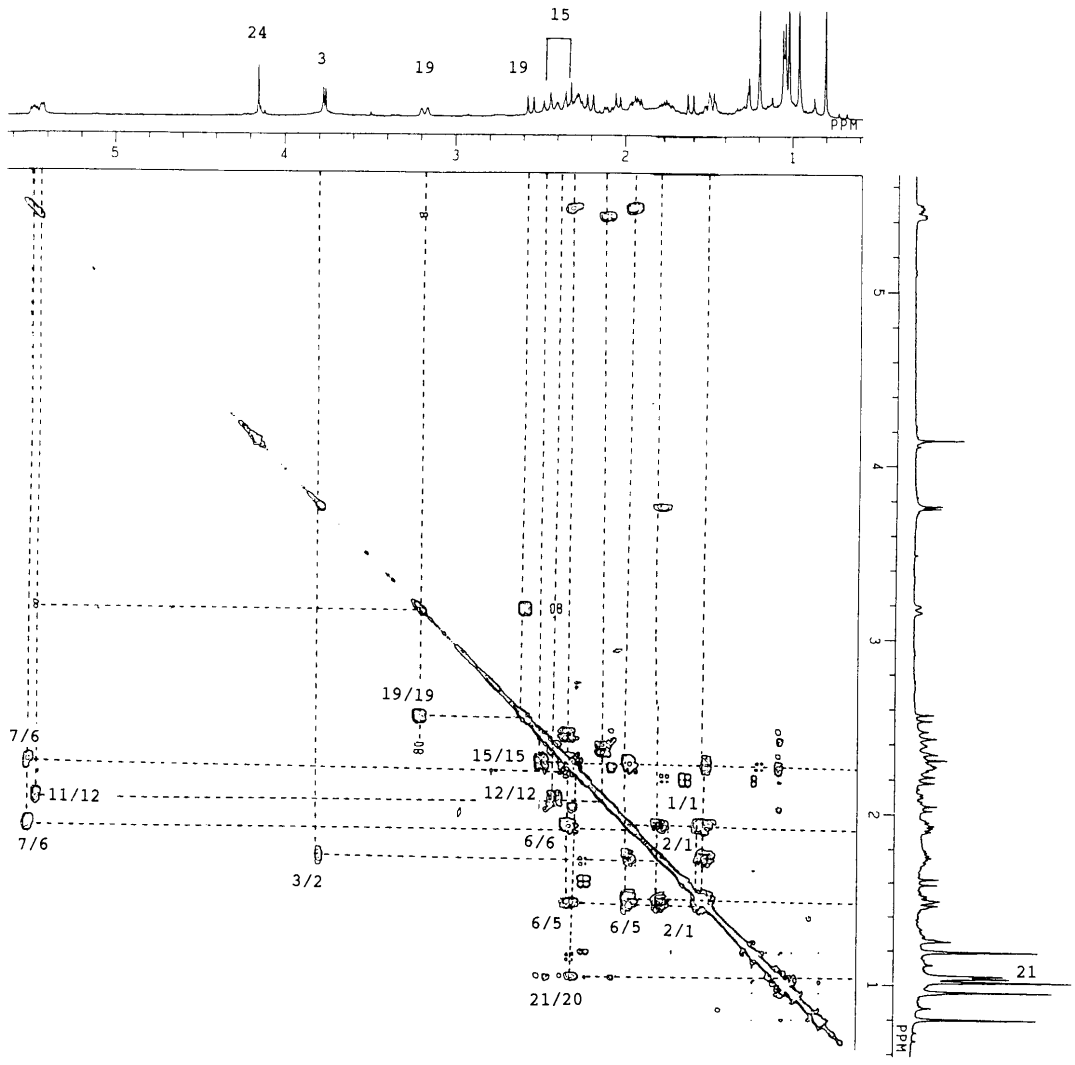

Fig. 4. ^{1}H–^{1}H COSY Spectrum of Compound **2c** in $CDCl_3$

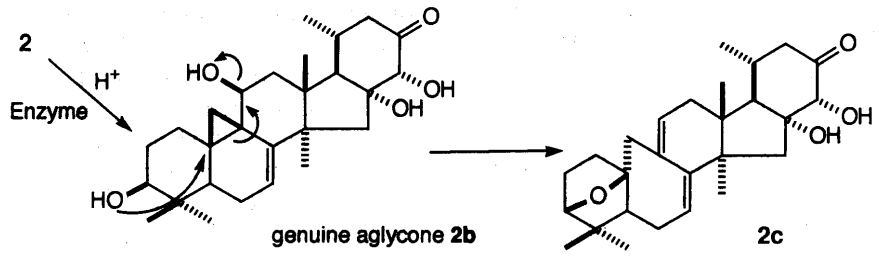

Chart 2

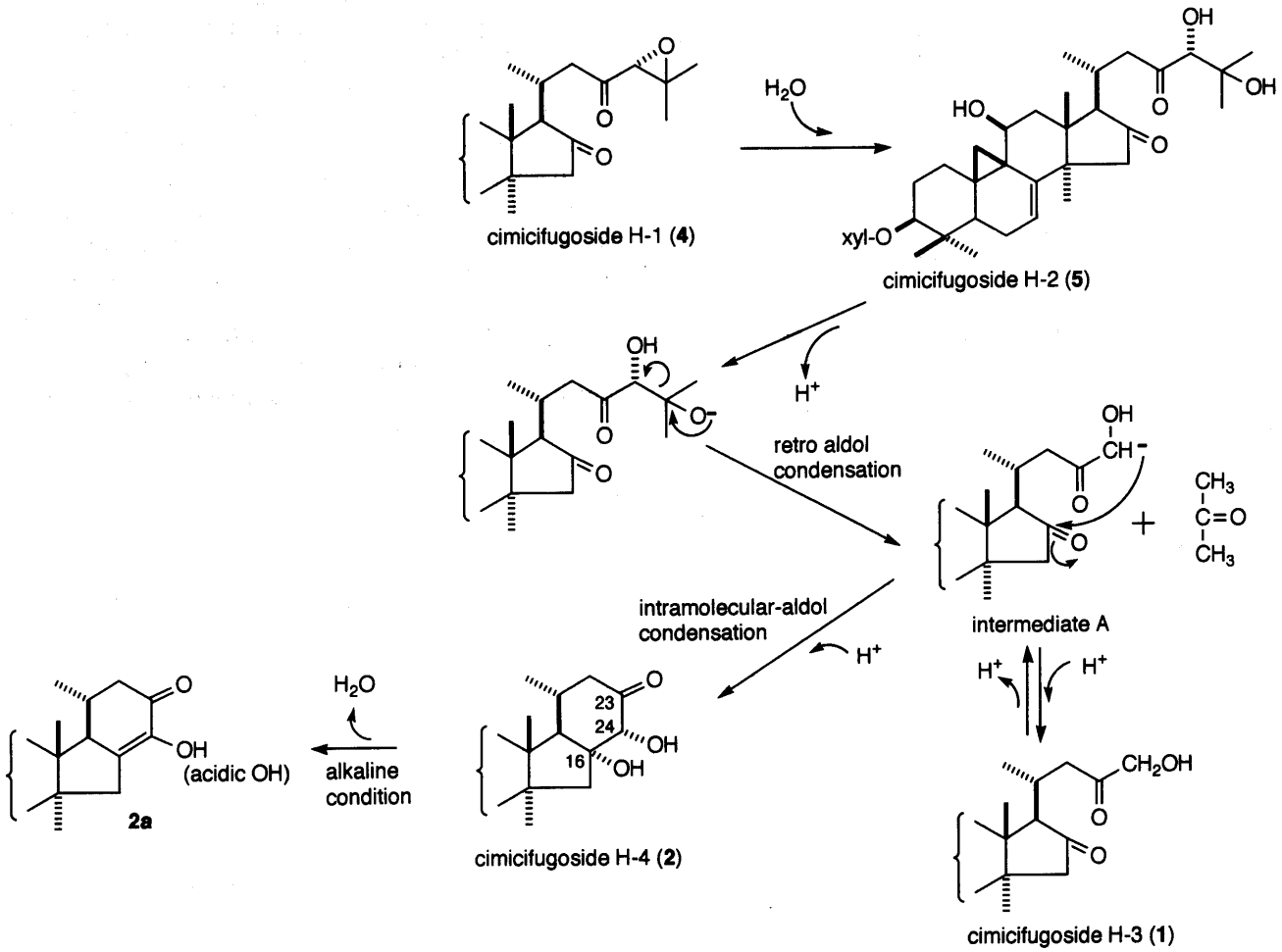

Chart 3

The stereochemistry of C-15 was elucidated on the basis of the information from a difference NOE experiment. Positive NOEs were observed among 18-H_3, 24-H_β and 15-H. From these results of the NOE experiment, the stereochemistry of the hydroxy group at C-15 was established to be α. The chemical shift due to 7-H in the ^{1}H-NMR spectrum showed a marked downfield shift owing to the introduction of 15α-OH into cimicifugoside H-4 (**2**), which is accounted for by the steric hindrance of 7-H and 15α-OH. The upfield shift of the signal due to C-28 in the ^{13}C-NMR spectrum is accounted for by the effect of steric congestion of C-28 and 15α-OH.

Thus, the structure of cimicifugoside H-6 (**3**) was established as 11β,15α,16α,24α-tetrahydroxy-3β-(β-D-xylopyranosyloxy)-25,26,27-trinor-9,19:16,24-dicyclolanost-7-en-23-one.

Six cimicifugoside H's have been found so far; two of them, H-5 and H-6, are 15-hydroxylated H-1 and H-4, respectively. It has already been reported that cimicifugoside H-1 (**4**) is convertible into cimicifugoside H-2 (**5**) under an acidic condition.[1] We investigated the transformation of **5** into cimicifugosides H-3 (**1**), H-4 (**2**) and the α-hydroxy enone (**2a**) under an alkaline condition as follows.

On treatment of cimicifugoside H-2 (**5**) with sodium hydrogencarbonate in methanol–water (1:1), **5** was rapidly converted into cimicifugoside H-3 (**1**, minor) and cimicifugoside H-4 (**2**, major). After 8 h, all of **5** had changed into **2**. Finally, **2** changed into the α-hydroxy enone (**2a**). These transformations of cimicifugosides were followed by TLC. This series of reactions under the alkaline condition can be explained as follows (Chart 3): firstly, cimicifugoside H-2 (**5**) affords acetone and an intermediate A (retro aldol condensation). Addition of a proton to the carbanion at C-24 affords cimicifugoside

H-3 (**1**). On the other hand, the carbanion attacks the ketonic carbon at C-16 to form a new cyclohexanone ring (intramolecular aldol condensation), affording cimicifugoside H-4 (**2**) directly. Cimicifugoside H-3 (**1**) is convertible into **2** *via* intermediate A. Finally, **2** changes into the α-hydroxy enone (**2a**) by dehydration. The one-way transformation of **2** into **2a** under the alkaline condition is probably due to ionization of the enol-acidic hydroxyl at C-24 of **2a**.

We think that the biogenetic route of these cimicifugoside H's would proceed from cimicifugoside H-1 to other cimicifugosides as described above. If so, cimicifugoside H-1 (**4**) is the parent glycoside of cimicifugosides H-2, H-3 and H-4.

After we had reported on cimicifugosides H-1, H-3 and H-4,[9] Kadota and his co-workers reported the isolation of some cyclolanosterol glycosides, having $\Delta^{7(8)}$ and a hydroxy group at C-11, from *Cimicifuga foetida*.[13] The genin part of cimicidanol-3-*O*-arabinoside (**6**)[13] is identical with that of cimicifugoside H-1 (**4**)[1]; different sugar moieties of the two are inferred from the chromatographic data on the sugars, although their ^{13}C-NMR spectral data are very similar. They also reported cimicidol-3-*O*-β-xyloside, foetidinol-3-*O*-β-xyloside and 15α-hydroxyfoetidinol-3-*O*-β-xyloside as white powders or slightly yellow powders: the melting points were not given. But the structures proposed for their three compounds are identical with those of our crystalline cimicifugosides H-2 (**5**), H-4 (**2**) and H-6 (**3**),[1,9] respectively.

Experimental

The instruments used for obtaining physical data and the conditions for chromatography were the same as described in the preceding paper.[1] Silica gel (Silica gel 60, Merck) and ODS (YMC Gel ODS-A120-230/70, Nishio) were used for column chromatography. Preparative HPLC was performed using an ODS column (Capcell Pak-C_8, Shiseido Co., Ltd., 6.0 × 150 mm; detector, refractive index).

Isolation of Compounds 1, 2 and 3 The procedures for extraction of fractions C_3 and D_5 are described in the preceding paper.[1] Fraction C_3 gave **2** (1 g). Fraction D_5 was subjected to column chromatography on silica gel with $CHCl_3$–MeOH (8 : 1) to give fr. E_1—E_4. Fraction E_2 was rechromatographed on an ODS (RP-18) column with MeOH–H_2O (2 : 1) to give fr. F_1—F_5. Fraction F_3 afforded **1** (20 mg). Fraction E_3 was subjected to ODS column chromatography (RP-18) with MeOH–H_2O (2 : 1), rechromatographed on a silica gel column with $CHCl_3$–MeOH (8 : 1) and purified on a Sephadex LH-20 column with MeOH to give **3** (10 mg).

Properties of Cimicifugoside H-3 (1) Colorless needles (MeOH), mp 249—251 °C. $[\alpha]_D$ −22.3° (*c* = 0.4, $CHCl_3$–MeOH, 1 : 1). Alkaline blue tetrazolium reaction (on TLC): positive (blue). IR ν_{max} (KBr) cm^{-1}: 3500—3300, 1040 (OH), 1730, 1710 (C=O). Positive FAB-MS *m/z*: 577 $[M+H]^+$. Positive HRFAB-MS Calcd for $C_{32}H_{49}O_9$ *m/z*: 577.3377. Found: 577.3382. EI-MS *m/z*: 558 (M^+-H_2O), 444 $(M^+-C_5H_8O_4)$. ^{1}H- and ^{13}C-NMR: Tables 1 and 2.

Enzymatic Hydrolysis of 1 To a solution of **1** (71.6 mg) in MeOH (5 ml) was added 0.003% AcOH (about 20 ml) under stirring to adjust the pH to 5. Cellulase T [Amano] 4 (from *Trichoderma viride*) (400 mg) was added and the mixture was stirred for 7 d at 37 °C. The MeOH was removed *in vacuo*, then the reaction mixture was shaken with EtOAc. The EtOAc layer was washed with water, dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The residue was chromatographed on silica gel with benzene–AcOEt (1 : 2), and subjected to preparative HPLC to afford a diketonic genin (**1a**) as a white amorphous powder. EI-MS *m/z*: 444 (M^+), 426 (M^+-H_2O). HREI-MS Calcd for $C_{27}H_{40}O_5$ *m/z*: 444.2874. Found 444.2874. ^{1}H- and ^{13}C-NMR: Tables 1 and 2. Compound **1a** was easily converted into foetidinol (**2b**). The water-soluble part was passed through an Amberlite MB-3 column, and concentrated under reduced pressure to give xylose on TLC [sol. BuOH–AcOH–H_2O (6 : 1 : 2), *Rf* value: 0.38].

Properties of Cimicifugoside H-4 (2) Colorless needles (MeOH), mp 265—267 °C. Optical rotatory dispersion (ORD) (*c* = 1.0, $CHCl_3$–MeOH, 1 : 1) [α] (nm); −75.0° (589), −77.9° (577), −92.4° (546), −197.4° (435), −440.3° (365). Bitter taste. Alkaline blue tetrazolium reaction on TLC: positive (blue). IR ν_{max} (KBr) cm^{-1}: 3500—3350, 1040 (OH), 1720 (C=O). Positive FAB-MS *m/z*: 577 $[M+H]^+$. Negative FAB-MS *m/z*: 575 $[M-H]^-$. Positive HRFAB-MS Calcd for $C_{32}H_{48}O_9Na$ *m/z*: 599.3196. Found: 599.3208. EI-MS *m/z*: 558 (M^+-H_2O), 444 $(M^+-C_5H_8O_4)$. HREI-MS Calcd for $C_{27}H_{40}O_5$: 444.2876. Found: 444.2879. ^{1}H- and ^{13}C-NMR: Tables 1 and 2.

Alkaline Treatment of 2 A solution of **2** (30 mg) in 5% KOH–MeOH was allowed to stand overnight at room temperature. Usual work-up afforded an α-hydroxy enone (**2a**) (12.0 mg) as a white powder (acetone–MeOH, 1 : 1). Alkaline blue tetrazolium reaction: negative (no coloration). UV λ_{max} (MeOH) nm (ε): 273 nm (18800). Positive FAB-MS *m/z*: 559 $[M+H]^+$. EI-MS *m/z*: 408 $(M^+-C_5H_8O_4-H_2O)$. HREI-MS Calcd for $C_{27}H_{36}O_3$ *m/z*: 408.2667. Found: 408.2666. ^{1}H- and ^{13}C-NMR: Tables 1 and 2.

Enzymatic Hydrolysis of 2 To a solution of compound **2** (25.5 mg) in MeOH (3 ml) (about 50 ml) was added 1 N AcOH–1 N NaOAc buffer under stirring to adjust the pH to 5. Cellulase T [Amano] 4 (from *Trichoderma viride*) (50 mg) was added and the mixture was stirred for 2 d at 30 °C. The MeOH was removed *in vacuo*. The reaction mixture was shaken with EtOAc. The EtOAc layer was washed with water, dried over anhydrous Na_2SO_4 and concentrated. The residue was chromatographed on silica gel with benzene–EtOAc (1 : 1) to afford the genuine aglycone (**2b**), colorless needles (AcOEt), mp 260—261 °C, $[\alpha]_D$ −84.8°. EI-MS *m/z*: 444 (M^+). HREI-MS Calcd for $C_{27}H_{40}O_5$ *m/z*: 444.2875, Found: 444.2870, and an artifactual genin (**2c**), colorless needles (hexane–MeOH), mp 232—233 °C. Positive FAB-MS *m/z*: 427 $[M+H]^+$, 449 $[M+Na]^+$. UV λ_{max} ($CHCl_3$) nm (ε): 243 (ε = 18400), 249 (19700), 259 (sh, 14000), 284 (1210). Compound **2b** was identified as foetidinol by comparison of the data with those reported for the genin in the literature.[13] ^{1}H- and ^{13}C-NMR of **2b** and **2c**: Tables 1 and 2. The water-soluble part was treated with Amberlite MB-3, and concentrated under reduced pressure. The residue was subjected to TLC with BuOH–AcOH–H_2O (6 : 1 : 2), and identified as xylose. *Rf* value: 0.38.

Properties of 3 Colorless needles (MeOH), mp 275—276 °C. ORD (*c* = 0.4, $CHCl_3$–MeOH, 1 : 1) [α] (nm): −64.3° (598), −68.0° (577), −80.9° (546), −181.4° (435), −425.7° (365). Alkaline blue tetrazolium reaction on TLC: positive (blue). IR ν_{max} (KBr) cm^{-1}: 3500—3400 (OH), 1725 (C=O). Negative FAB-MS *m/z*: 591 $[M-H]^-$. Negative HRFAB-MS Calcd for $C_{32}H_{47}O_{10}$ *m/z*: 591.3169. Found: 591.3172. ^{1}H- and ^{13}C-NMR: Tables 1 and 2.

Alkaline Treatment of Cimicifugosides H-2 (5) and H-3 (1) a) To a solution of **5** in MeOH (about 1 ml) was added saturated aqueous $NaHCO_3$ (about 1 ml). The solution was allowed to stand at room temperature. The progress of the reaction was followed by TLC, and the transitional products were identified by TLC. TLC [HPTLC-Fertigplatten RP-8 $F_{254}S$ (Merck); solv., MeOH–H_2O (2 : 1); detection, 10% H_2SO_4 followed by heating, or alkaline blue tetrazolium reagent] *Rf* values: 0.29 (**2a**), 0.45 (**5**), 0.50 (**1**), 0.57 (**2**). After 1 h, unchanged. After 2 h, a small amount of **5** had changed into cimicifugosides H-3 (**1**, minor) and H-4 (**2**, major). After 4 h, about half of **5** had changed into **1** and **2**. After 8 h, all of **5** had changed into **1** (a little) and **2**. After 24 h, **1** and **2** had changed into an α-hydroxy enone (**2a**). b) To a solution of **1** in MeOH (about 1 ml) was added one drop of saturated aqueous $NaHCO_3$. The solution was allowed to stand at room temperature. The progress of the reaction was followed by TLC. TLC [HPTLC-Fertigplatten RP-18 $WF_{254}S$ (Merck), solv. MeOH–H_2O (7 : 3), detection; 10% H_2SO_4] *Rf* values: 0.40 (**2a**), 0.50 (**1**), 0.58 (**2**). Cimicifugoside H-3 (**1**) changed into cimicifugoside H-4 (**2**) slowly. After 8 h, about a quarter of **1** had changed into **2**. After 24 h, about a half of **1** had changed into **2**. And **2**, which was formed by transformation of **1**, changed into **2a** slowly.

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