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Quantitative comparisons of antitermite extractives in heartwood from the same clones of *Cryptomeria japonica* planted at two different sites

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Abstract The quantities of some extractives in the heartwood of 25 plus tree clones of Japanese cedar (*Cryptomeria japonica*) from two different sites were investigated to clarify the differences in antitermite properties in relation to clones and environmental factors. The measured compounds were cubebol, epicubebol, sandaracopimarinol, and ferruginol. The total amounts of extractives were calculated from gas chromatogram peak areas. The heartwoods from Tano (Tano Forest Research Station, Miyazaki University; 31° N, 131° E, 130 m asl) tended to contain more of the individual extractives and total extractives than those from Komenono (Komenono Forest Research Station, Ehime University; 33° N, 132° E, 700 m asl). There was a significant difference (calculated by analysis of variance) at the 1% level among clones at both sites in quantities of total and individual extractives. This result suggests that the qualities and quantities of heartwood extractives are largely affected by genetic factors. Spearman's rank correlation with the average of the extractive quantities at the sites was investigated. The correlation coefficients of total extractives, cubebol, epicubebol, sandaracopimarinol, and ferruginol were 0.61, 0.85, 0.76, 0.67, and 0.74, respectively. This result means that the order of the amounts of these compounds among the cultivars could be maintained at different sites. It is apparent that both environmental and genetic factors affected the quantities of these extractives in the heartwoods of Japanese cedar.

Key words Antitermite · Extractives · *Cryptomeria japonica* · Terpenoids · Clones

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Introduction

Japanese cedar (*Cryptomeria japonica* D. Don), which is endemic to Japan, is the major Japanese coniferous tree species and has been planted in large areas of the Japanese islands for a long time. There are many cultivars having characteristic properties, which have often been named after the regions in which they are grown.

Many cedar trees have been planted in several regions including the Kyushu region. Because the trees have been proliferated by cutting in Kyushu, it seems that the trees from this region hold the genetic features of the original tree.

Many studies have been made on the extractives in cedar heartwood, especially on norlignans and terpenoids. The effect of the composition of norlignans on the color of cedar heartwood has been studied.[1–4] Terpenoids in Japanese cedar generally contain more sesquiterpenes than terpenoids of *Pinus* species. It has been reported that terpenoids from different cedar cultivars differ in composition.[5–8] The outer part of heartwood of yakusugi, a cultivar of cedar, was found to contain more terpenoids than in the inner part of the heartwood.[9,10]

There is less interest in the quantitative differences in the heartwood extractives of cedar wood in relation to their practical use. In recent times, cedar wood has been kiln-dried to supply good quality timber. This process decreases the heartwood extractives and so reduces the resistance of the heartwood against termites and wood rot fungi.[11] For this reason, it is important to quantitatively investigate the variation of extractives in cedar heartwood, because the durability of wood generally depends upon its extractives. α-Cadinol and T-muurolol in the heartwood of *Chamaecyparis obtusa* Endl, saponins from *Ternstroemia japonica* Thunb, and taxifolin from *Larix leptolepis* Zieb. et Zucc are well known as feeding deterrents against termites.[12–14]

Some terpenoids from the heartwood of Japanese cedar inhibit termite feeding. Sandaracopimarinol from the heartwood of obi-sugi, a cultivar of Japanese cedar, as well as cubebol and epicubebol from the black heartwood of the cedar, have been reported to be termiticidal components.[15,16] Ferruginol, commonly found in the heartwood, not only deterred feeding of the Japanese termite, *Reticulitermes speratus*, but also inhibited growth of wood-decaying fungi.[17,18] Moreover, an increase in the amount of this compound in the heartwood was correlated with a decrease in the quantity of wood eaten by *R. speratus*.[11]

Of interest is the variation in the quantities of extractives among the cultivars or the groups of trees planted at different sites. However, this variation has been little reported. In this study, the quantitative features of the extractives from the same clonal group of Japanese cedar planted at two different sites were analyzed and statistically evaluated to clarify the effects of the cultivars and the planting site environments.

Experimental

Materials

Samples of plus tree clones were harvested from plus tree clonal test forests at the Komenono Forest Research Station of Ehime University (located at 33° N, 132° E) and the Tano Forest Research Station of Miyazaki University (located at 31° N, 131° E). The altitude of the Komenono Forest Research Station was 700 m and that of the Tano Forest Research Station was 130 m. Mean annual precipitation for both sites was reported as about 2000 mm. The soil type of the two sites was BD. The annual mean temperature of Komenono is 10.8°C, and that of Tano is 16.0°C.[19] Twenty-five plus tree clones selected from all over the Kyushu region were planted in each stand in 1971. The names of these cultivars, together with brief symbols and their sample numbers, are shown in Table 1. Sample logs were harvested at Komenono in November 2002 and at Tano in November 2003. Cross-sectional samples about 10 cm thick were cut at breast height (1.2 m) and air-dried at room temperature. The outer parts with five annual rings of heartwood were cut from these samples and powdered using a grinder. The wood meal passing through a 1-mm mesh sieve was used for extraction.

Table 1. Tested plus tree clones and sample numbers

Common name of cultivar	Symbol	Sample number	
		Komenono	Tano
Higasiusuki 12	a	4	7
Ken ohita 5	b	4	4
Ken satsuma 5	c	4	3
Fukuoka syo 1	d	4	3
Ken aira 6	e	4	7
Ken aira 26	f	4	0
Ken higasiusuki 4	g	4	3
Ken aira 25	h	4	3
Ken kagoshima 1	i	4	3
Ken takeda 4	j	4	4
Ken takeda 6	k	4	7
Ken nagasaki 1	l	4	3
Ken fujitu 14	m	4	1
Ken miyakonojou 5	n	4	2
Ken takeda 9	o	3	5
Ken aira 15	p	4	7
Ken takeda 15	q	4	4
Ken aso 1	r	4	2
Ken aira 21	s	4	7
Ken saga 3	t	4	4
Aya syo 1	u	4	2
Ohnejime syo 1	v	4	3
Miyazaki syo 4	w	4	0
Ken fuwa 11	x	4	7
Ohkuchi syo 2	y	4	1

Extraction

One gram of each meal was extracted with 20 ml of a mixed solvent (*n*-hexane/benzene = 1/1) for 48 h at room temperature. The filtered extractive solutions were analyzed by gas chromatography without condensation.

Chemical analyses of extractives

The extractive solutions were analyzed by gas chromatography (GC-14B, Shimadzu, Kyoto, Japan). The analytical conditions were: TC-Wax column (0.25 mm × 30 m, i.d. 0.25 μm, GL-Sciences, Tokyo, Japan); initial temperature 60°C; initial time 1.0 min; programmed temperature 2.0°C/min; final temperature 240°C; final time 70 min; He carrier gas; detector temperature 245°C, injector temperature 210°C; flame ionization detection (FID).

The major constituents estimated from previous studies as antitermite compounds[11,15,16] were fractionated by thin-layer chromatography with a mixed solvent (*n*-hexane and benzene) and were analyzed by gas chromatography-mass spectrometry (GC-MS; GC-MS QP-5000, Shimadzu). The analytical conditions were as follows: DB-1 column (0.25 mm × 30 m, i.d. 0.25 μm, J&W Scientific, Florida, USA); injector temperature 250°C; initial temperature 60°C; initial time 5.0 min; programmed temperature 2.0°C/min; final temperature 280°C; final time 20 min; He carrier gas; mass temperature 280°C.

The compounds were identified by comparing the fragmentation patterns obtained with those of the corresponding compounds in previous studies.[20] The mass fragmentation patterns are summarized as: epicubebol (*m/z*): 222 [M^+] (3.3), 207 [M^+–15] (74.6), 189 (7.5), 179 (7.9), 161 (100), 151 (10.3), 135 (24.3), 121 (49.3), 105 (77.4), 93 (53.3), 81 (65.2); cubebol (*m/z*): 222 [M^+] (2.5), 207 [M^+–15], 189 (0.8), 179 (16.4), 161 (100), 151 (10.3), 135 (24.6), 119 (68.3), 105 (89.0), 93 (59.6), 81 (73.3); sandaracopimarinol (*m/z*): 288 [M^+] (18.6), 273 [M^+–15] (14.2), 257 (100), 241 (2.5), 227 (1.2), 215 (2.8), 201 (8.7), 187 (10.0), 175 (14.7), 161 (30.1), 147 (33.5), 135 (54.2), 121 (74.7), 107 (63.4), 91 (65.1), 81 (79.1); ferruginol (*m/z*): 286 [M^+] (84.1), 271 [M^+–15] (98.3), 255 (1.8), 243 (5.6), 229 (17.8), 215 (17.2), 201 (48.8), 189 (93.9), 175 (83.8), 159 (28.8), 149 (39.9), 141 (14.6), 133 (26.2), 123 (1.9), 115 (16.6), 91 (16.2), 83 (18.4). The assignment of the chromatogram peaks was based on their retention times.

The quantities of extractives were determined by comparison of peak areas with tridecane internal standard. The calculated values of the extractives were statistically analyzed with a statistical software package (Statcell, OMS, Saitama, Japan).

Results and discussion

The quantities of cubebol, epicubebol, sandaracopimarinol, and ferruginol extractives in Japanese cedar heartwood were investigated in this study. These compounds were previously reported to have antitermite activity.[11,15,16] In addition, the total amounts of *n*-hexane and benzene extractives estimated as the main neutral terpenoids were calculated by the sum of peak areas of the chromatogram rejecting the area given by the solvents.

Figure 1 shows the concentrations of total extractives and of cubebol, epicubebol, sandaracopimarinol, and ferruginol contained in the heartwoods. The group from Tano tended to have higher concentrations of total and individual extractives than that from Komenono. About half of the individuals from Tano had a concentration of total extractives of 4.5% or more while most of the Komenono trees had less than 3.0%. The individual extractives showed the same trend as that of the total. These results indicate that the planting environment affected the concentration of extractives in the heartwood.

The differences between the two forests within each cultivar were investigated. The contents of the neutral *n*-hexane extracts are shown in Table 2. The major components were found to be neutral terpenoids. The cultivars tested were a–x, with the exceptions of f, m, w, and y, which had fewer than two replications at Tano. The results are illustrated in Fig. 2 for the concentration of total extractives and of cubebol, epicubebol, sandaracopimarinol, and ferruginol.

The mean concentrations of total extractives in the heartwood of the cultivars from Tano were 1.6% to 14%, and 0.65% to 6.6% for those from Komenono (Fig. 2a). The concentrations in heartwood samples from Tano were higher than those from Komenono in almost the cultivars. However, the trends of increasing concentrations differed among cultivars between the two sites.

The mean concentrations of cubebol in the heartwood of the cultivars from Tano were 0.20% to 6.3%, and 0.031% to 1.5% for those from Komenono (Fig. 2b). Mean concentrations of epicubebol were 0.15% to 1.6% for Tano and 0.01% to 0.61% for Komenono (Fig. 2c). The concentrations of cubebol and epicubebol in each cultivar from Tano were higher than those from Komenono. The concentrations of cubebol of the plus tree t showed extreme amounts at both sites. On the other hand, there was little difference in concentrations of cubebol in samples n, o, and u from both sites. The same tendency was observed for the concentration of epicubebol. A large amount of cubebols comprising cubebol and epicubebol was reported in some heartwood

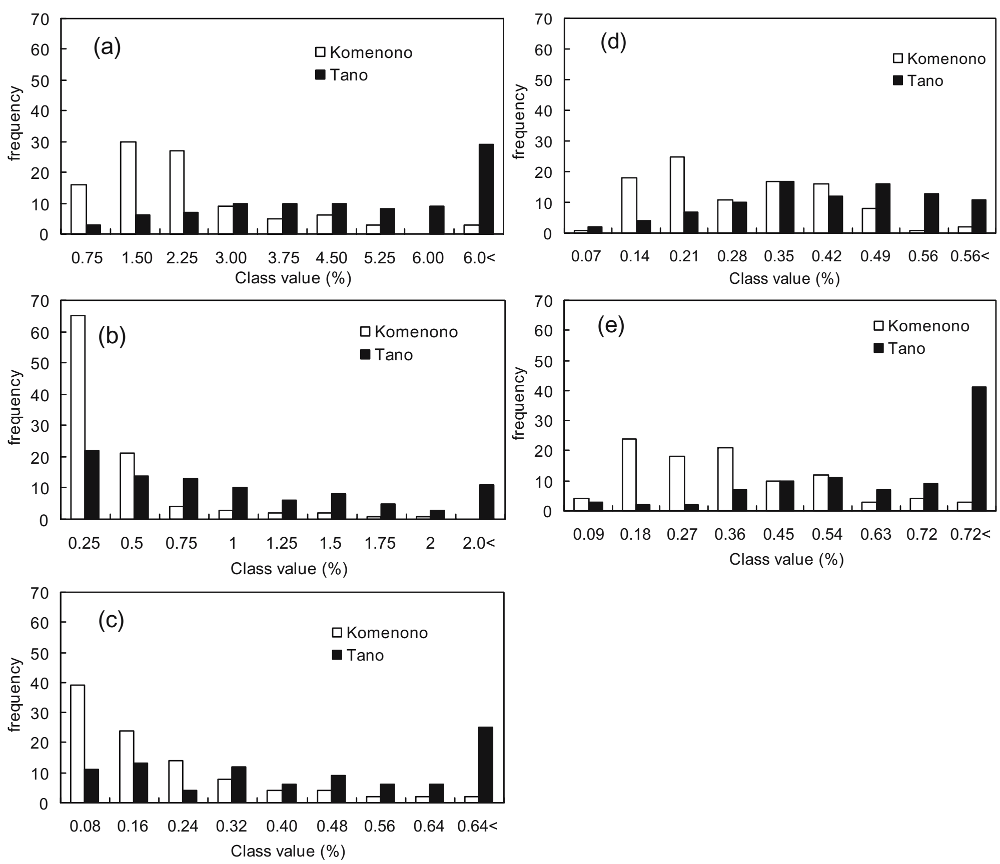

Fig. 1a–e. Frequency distributions of extractives in the heartwood of *Cryptomeria japonica* trees at the planting sites of Tano and Komenono. **a** Total combined neutral terpenoids; **b** cubebol; **c** epicubebol; **d** sandaracopimarinol; **e** ferruginol

Table 2. Mean contents of each compound in *n*-hexane extractives

Cultivar	Cubebol		Epicubebol		Sandaracopimarinol		Ferruginol	
	Komenono	Tano	Komenono	Tano	Komenono	Tano	Komenono	Tano
a	13.6	14.0	10.3	9.4	11.7	5.4	14.3	11.9
b	16.2	15.6	12.3	8.4	13.0	8.3	20.1	17.0
c	14.0	12.7	7.9	8.7	12.7	7.0	17.7	12.3
d	9.5	23.2	5.8	12.5	10.5	15.8	8.4	7.3
e	17.0	19.1	11.1	11.1	12.0	17.3	18.4	15.0
f	2.5	NS	1.7	NS	17.3	NS	17.6	NS
g	22.5	26.9	12.8	11.9	13.4	6.7	12.9	10.0
h	2.0	14.0	1.4	6.9	17.1	11.1	18.6	14.0
i	2.9	3.7	2.0	2.8	19.0	7.3	18.0	9.0
j	6.8	9.2	5.0	5.1	19.5	15.3	25.6	24.8
k	8.8	12.7	5.9	6.2	17.3	11.7	25.4	18.9
l	15.5	14.6	9.5	9.1	16.3	6.3	20.4	11.0
m	3.3	10.5	2.8	6.2	14.0	6.4	11.3	7.9
n	14.5	18.1	10.0	10.4	11.9	8.3	9.5	9.0
o	5.4	11.2	3.5	5.6	20.6	12.6	26.8	21.9
p	4.9	14.8	4.0	7.8	23.0	13.2	21.1	15.2
q	14.6	19.3	9.7	9.3	13.8	6.5	16.2	13.0
r	3.8	13.8	2.9	7.6	13.1	9.1	11.8	10.7
s	26.0	24.5	11.0	8.8	9.2	6.1	17.4	14.2
t	23.8	44.4	9.4	11.6	6.7	3.8	7.8	8.9
u	2.8	1.3	2.2	1.1	21.2	16.1	42.7	41.1
v	13.8	12.9	8.8	8.9	14.1	8.0	11.2	9.4
w	9.1	NS	6.7	NS	14.9	NS	18.6	NS
x	27.3	16.4	15.2	7.9	7.4	4.4	20.9	16.7
y	6.3	13.1	4.6	7.6	20.8	12.2	31.3	20.3

Content given as percentages
NS, No sample

Table 3. Analysis of variance for total extractives and individual terpenoids

Factor	Sum of deviation square	Degrees of freedom	Mean square	*F*	*P*
Total extractives					
Sum of square	1786.4	172			
Sites	465.9	1	470	121.7	2.05×10^{-20}
Clones	711.8	20	36	9.3	8×10^{-17}
Interaction	107.2	20	5.4	1.4	0.13
Error	501.6	131	3.8		
Cubebol					
Sum of square	210.9	172			
Sites	27.0	1	27	89.3	1.79×10^{-16}
Clones	107.9	20	5.4	17.8	3.11×10^{-28}
Interaction	36.2	20	1.8	6.0	6.21×10^{-11}
Error	39.7	131	0.30		
Epicubebol					
Sum of square	22.8	172			
Sites	4.4	1	4.4	76.2	1.04×10^{-14}
Clones	8.9	20	0.45	7.8	3.10×10^{-14}
Interaction	2.0	20	0.10	1.8	0.03
Error	7.5	131	0.057		
Sandaracopimarinol					
Sum of square	4.1	172			
Sites	0.7	1	0.067	84	8.84×10^{-16}
Clones	2.1	20	0.10	12.8	4.28×10^{-22}
Interaction	0.3	20	0.016	2.0	0.01
Error	1.0	131	0.008		
Ferruginol					
Sum of square	19.9	172			
Sites	6.1	1	6.1	152.7	9.8×10^{-24}
Clones	7.3	20	0.37	9.2	9.72×10^{-17}
Interaction	1.3	20	0.063	1.6	0.06
Error	5.2	131	0.040		

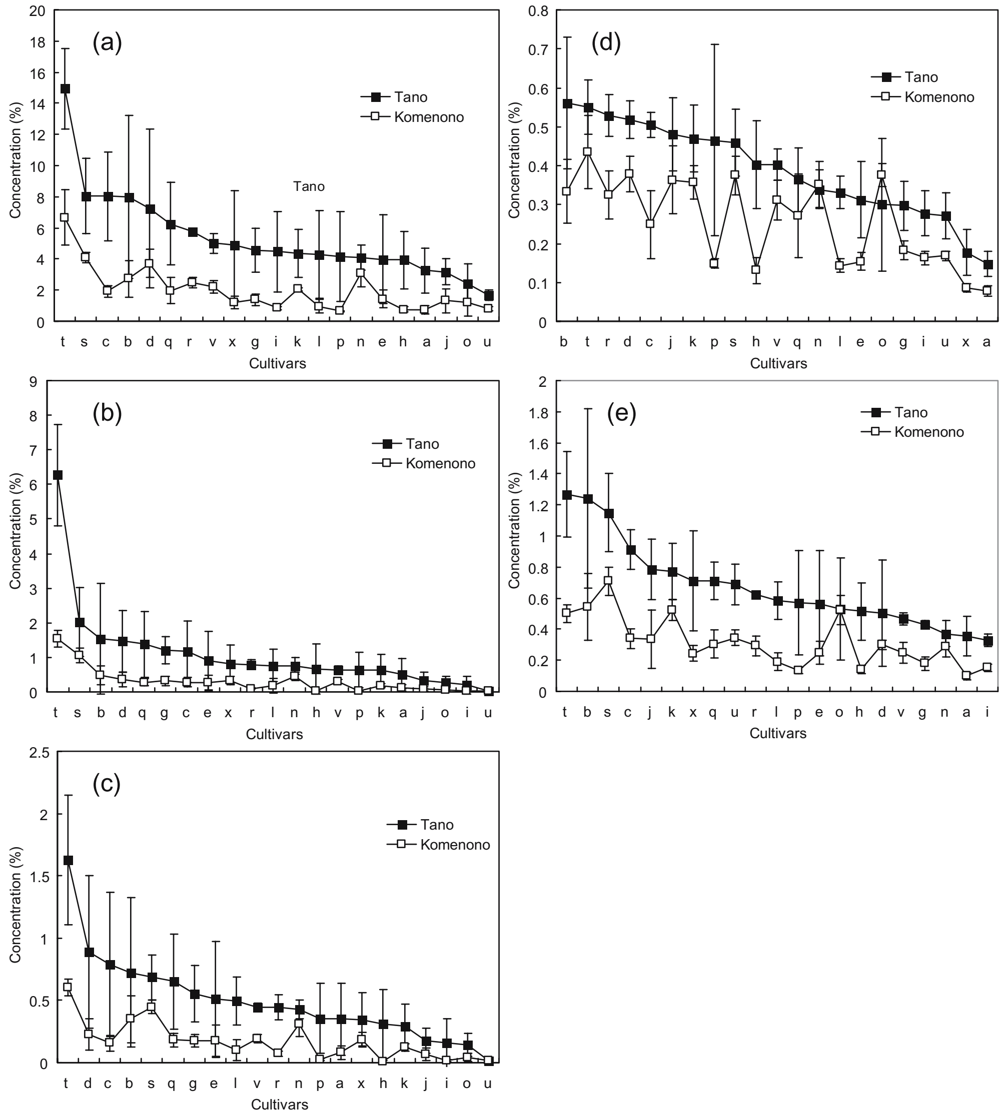

Fig. 2a–e. Concentrations of extractives in cultivars from the planting sites of Tano and Komenono. **a** Total combined neutral terpenoids; **b** cubebol; **c** epicubebol; **d** sandaracopimarinol; **e** ferruginol. *Error bars* show standard deviations

samples of Japanese cedar.[9] These extreme differences of concentration of cubebols were found to be affected by both genetic and environmental factors.

The mean concentrations of sandaracopimarinol in the cultivars at Tano ranged from 0.15% to 0.56%, and from 0.078% to 0.38% at Komenono (Fig. 2d). The mean value for ferruginol at Tano ranged from 0.33% to 1.3%, and from 0.098% to 0.71% at Komenono (Fig. 2e). Although the concentration distribution of sandaracopimarinol was similar to that of ferruginol, no complete equality was observed. With these compounds having similar chemical structures, it is likely that their biosynthesis is linked.

The results of analysis of variance (ANOVA) for the concentrations of extractives in relation to cultivars and planting location are shown in Table 3; significant differences among the cultivars and the two locations are apparent. The interaction between both cultivars and locations was significant for cubebol, epicubebol, and sandaracopimarinol (95% confidence level). However, the values of mean square among clones were more than three times larger than the values of the interaction. These results of ANOVA suggest that the effects of the interaction were small but some clones showed increased terpenoid levels in response to a strongly affected environment.

Each mean value of the concentration of extractives in cultivars was ordered within a planting site according to Spearman's analysis. The results show that the values of Spearman's ρ for concentrations of total extractives, cubebol, epicubebol, sandaracopimarinol, and ferruginol were 0.61, 0.85, 0.76, 0.67, and 0.74, respectively. These values are closer to 1.0 than 0. Therefore, it is suggested that the order of concentrations of these components among cultivars could be easily maintained even in different planting sites.

These results indicate that both genetic and environmental factors affected the concentrations of neutral terpenoids among extractives in Japanese cedar heartwood. Tamura et al.[21] reported that genetic and planting location factors affected yields of the alcohol–benzene extractives from the heartwood of Japanese cedar. Most of the alcohol–benzene extractives in the heartwood of the cedar are presumed to consist of terpenoids and phenolics such as norlignans. Therefore, it is suggested that the yields of the phenolics in the heartwood may also differ significantly, because significant differences in total yields of alcohol–benzene extractives and of the terpenoids have been found among the cultivars.

There is no information on what environmental factors cause differences in the amounts of these heartwood terpenoids. Some soil components may increase concentrations of extractives, because black heartwood of *C. japonica*, which has more extractives than the red heartwood, contains a high level of potassium ions.[16,22,23] Other environmental factors may also affect the concentrations of the extractives in the heartwood. If the potassium ion level in the trees affects the amounts of these terpenoids, high atmospheric temperature will enhance the production of terpenoids because of increased transpiration. This point may agree with the measured temperatures.[19] On the other hand, there was no correlation between growth traits (tree height and diameter at breast height) and terpenoids investigated in this study. Studies on the effects of environmental factors at planting sites may yield more useful information for utilizing Japanese cedar wood.

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