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JUVENILE–ADULT CHEMICAL DIMORPHISM IN FOLIAGE OF *DACRYDIUM BIFORME**

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Key Word Index—*Dacrydium biforme*; Podocarpaceae; infraspecific variation; juvenile–adult dimorphism; diterpenes; phyllocladene; 8α-isopimara-9(11),15-diene; sesquiterpenes; (*Z*)-5-ethylidene-2(5H)-furanone.

Abstract—Phyllocladene was the major diterpene in 15 specimens of *Dacrydium biforme* adult foliage. The major sesquiterpenes, which vary considerably in level from tree to tree, have been identified. Juvenile and adult foliage extracts from the same trees differed greatly. 8α-Isopimara-9(11),15-diene was the major diterpene in juvenile foliage. (*Z*)-5-Ethylidene-2(5H)-furanone was an artefact of steam-distillation of juvenile leaves.

INTRODUCTION

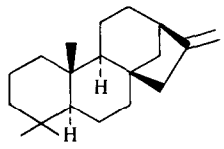
Dacrydium biforme (Hook.) Pilger is a tree endemic to the North, South and Stewart Islands of New Zealand [2, 3]. The name *biforme* refers to the sharply contrasting forms of the juvenile and adult foliage [4]. The transition from the linear juvenile leaves (10–20 mm) to the scale-like adult (1–2 mm) leaves is very abrupt, though reversion to juvenile foliage is common in adult plants [3–5]. Quinn suggests that this species should be treated as *Halocarpus biformis* (Hook.) C. J. Quinn, sharing this new genus with two other species endemic to New Zealand, *D. bidwillii* and *D. kirkii* [5].

The terpenes in the foliage of *D. biforme* have been investigated by Corbett and Wong [6], who identified phyllocladene (**1**) as the major diterpene, as did two other groups of investigators [7, 8]. A trace of isophyllocladene (**2**) has also been detected by GC [7]. In view of our discovery of major infraspecific variations of diterpene levels in the foliage of *D. cupressinum* [9] and *D. intermedium* [1], such apparent uniformity merited further investigation.

RESULTS AND DISCUSSION

Adult foliage components

Extractions and GC analyses of 15 samples of adult *D. biforme* foliage, from two widely separated sites, largely confirmed the findings of Corbett and Wong [6] (see Tables 1 and 2). Myrcene (**3**) was the major monoterpene in each extract. The major sesquiterpenes were α-longipinene (**4**), longifolene (**5**), caryophyllene (**6**), germacrene D (**7**), bicyclogermacrene (**8**) and δ-cadinene (**9**). Sesquiterpene levels varied greatly from tree to tree (Table 1), in a manner similar to the situation in *D. cupressinum* [10]. Phyllocladene (**1**) was the major diterpene in each tree (Table 2), along with isophyllocladene (**2**), isokaurene (**10**) and kaurene (**11**) which were identified by their GC retention indices [11]. This uniformity of diterpene compositions of adult *D. biforme* foliage samples contrasts dramatically with the great variations in levels found in *D. cupressinum* [9] and *D. intermedium* [1].



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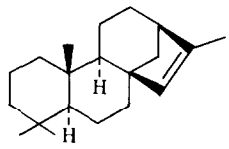

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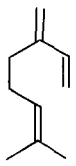

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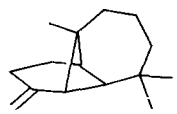

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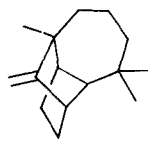

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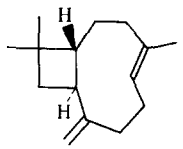

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*Part 4 in the series "Foliage Components of New Zealand Gymnosperms". For Part 3 see ref. [1].

Table 1. Levels of monoterpenes, sesquiterpenes and furanone in *Dacrydium biforme* foliage samples*

Compound‡	Tree No.†														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Myrcene	4.5	3.4	3.4	4.1	2.3	2.1	3.0	17.0	11.6	6.1	9.5 (0.8)	3.7 (0.2)	3.6 (0.3)	3.5 (0.1)	7.6 (0.9)
Furanone (**19**)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0 (1.7)	0.0 (1.2)	0.2 (1.6)	0.0 (4.0)	0.0 (2.9)
α-Longipinene	0.5	0.3	0.6	0.5	0.3	0.3	0.5	1.6	1.1	1.1	0.0 (0.0)	0.0 (0.0)	1.1 (0.2)	0.0 (0.0)	0.0 (0.0)
Longifolene	3.5	3.7	4.9	3.6	2.0	2.3	3.2	10.6	14.2	8.0	0.0 (0.0)	0.0 (0.0)	8.4 (2.0)	0.0 (0.0)	0.0 (0.0)
Caryophyllene	0.9	0.4	0.1	0.1	0.0	0.1	0.4	0.1	1.5	0.0	0.1 (0.0)	0.0 (0.0)	0.1 (0.0)	0.1 (0.0)	0.0 (0.0)
Germacrene D	1.5	1.2	0.4	1.1	0.5	1.0	1.0	1.2	2.7	1.1	6.8 (1.0)	0.9 (0.1)	2.2 (0.5)	4.2 (0.6)	5.2 (1.7)
Bicyclogermacrene	0.7	0.6	0.4	0.4	1.0	0.5	0.6	3.4	2.4	1.6	3.4 (1.2)	4.2 (1.1)	1.2 (1.0)	2.3 (0.8)	1.7 (1.6)
δ-Cadinene	0.9	0.9	0.8	1.0	0.4	0.7	0.7	2.6	2.5	1.7	2.6 (0.4)	1.8 (0.3)	1.0 (0.2)	1.6 (0.6)	2.0 (0.6)

*Peak areas are relative to octadecane internal standard.
†Figures in parentheses are for juvenile samples.
‡Compounds are listed in order of increasing retention index on SE-30.

Table 2. Levels of diterpenes in *Dacrydium biforme* foliage samples*

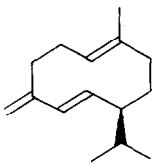
Compound‡	Tree No.†														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Isopimaradiene (**16**)	0.5	0.7	0.4	1.7	0.0	2.0	12.0	1.8	1.0	4.1	4.6 (70.3)	2.8 (59.9)	2.4 (64.3)	3.0 (47.1)	0.8 (0.6)
Sandaracopimaradiene	3.8	0.0	3.9	0.0	0.0	0.0	0.0	0.0	3.4	0.0	4.5 (9.6)	4.7 (8.1)	4.1 (9.4)	6.7 (10.8)	6.2 (6.1)
Isophyllocladene	15.1	17.1	13.7	15.7	21.9	15.9	25.0	19.1	14.2	22.5	2.0 (7.6)	2.7 (9.5)	7.6 (4.9)	7.0 (2.7)	12.2 (11.3)
Isokaurene	1.1	1.8	2.2	1.5	2.3	1.3	2.6	2.5	0.0	2.2	4.0 (1.7)	1.7 (0.9)	3.4 (1.6)	2.3 (1.8)	3.4 (2.5)
Phyllocladene	71.9	68.2	71.4	64.6	64.0	68.1	48.9	61.9	70.4	61.1	74.7 (7.5)	74.1 (15.3)	70.6 (14.4)	68.9 (26.6)	65.6 (66.3)
Kaurene	4.2	4.7	4.5	5.6	4.9	4.0	2.5	3.7	6.1	3.2	3.3 (0.5)	3.5 (1.0)	2.7 (0.6)	4.7 (1.6)	4.0 (3.1)

*Peak areas are given as % of total diterpenes.
†Figures in parentheses are for juvenile samples.
‡Compounds are listed in order of increasing retention index on SE-30.

Juvenile foliage components

GC analyses showed the same mono- and sesquiterpenes in juvenile foliage, but very different diterpene compositions (Table 2). Phyllocladene (**1**) was isolated from juvenile foliage, but only as a minor component (Table 2), as was sandaracopimaradiene (**12**). The major diterpene did not have the same GC retention indices as any of the common diterpene hydrocarbons [11].

^{1}H and ^{13}C NMR showed that the unknown was tricyclic, with a vinyl group and a trisubstituted double bond. Under mild acid conditions it rapidly rearranged to isopimara-8,15-diene (**13**), whilst longer acid treatment gave rimua-5(10),15-diene (**14**). The proton on the trisubstituted double bond was vicinally coupled, so the unknown had an isopimarane skeleton with a 7,8- or 9,11-double bond. A 7,8-double bond was ruled out by the mass spectrum, which did not contain the *m/z* 148, 133 and 109 peaks characteristic of isopimara-7,15-diene (**15**) [12]. Two possibilities remained: 8α-isopimara-9(11),15-diene (**16**) or the 8β-epimer (**17**). The latter has been synthesized [13] and had a similar mass spectrum but different ^{1}H NMR spectrum to the unknown, which is therefore 8α-isopimara-9(11),15-diene (**16**). A compound with similar ^{1}H NMR and IR spectra and GC retention was isolated from the foliage of *Chamaecyparis nootkaten-*



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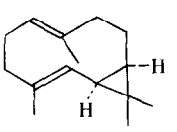

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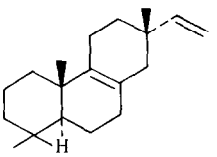

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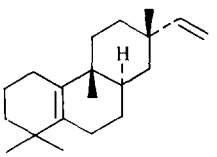

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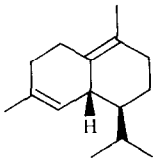

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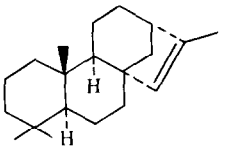

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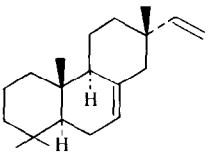

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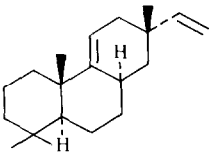

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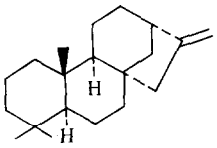

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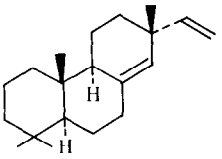

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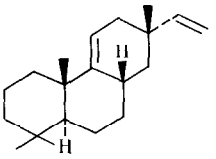

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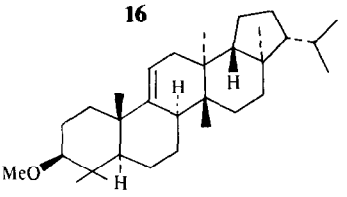

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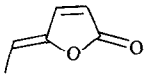

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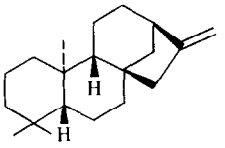

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sis (D. Don) Spach (Cupressaceae) [14], but was not fully characterized.

The ^{13}CNMR spectrum of 8α-isopimara-9(11),15-diene (**16**) contained several unusual resonances when compared with assigned spectra of pimara- and isopimaradienes [15], and podocarpenes and podocarpanes [16]. These confirmed the structural assignment. Models indicated that ring B would adopt a twist boat conformation rather than the usual twist chair, a structural feature shared by the triterpene arundoin (**18**). The ^{13}CNMR spectrum of this compound has recently been assigned [17], and confirms the unusually high-field resonances of C-5 and C-7, and the unusually low-field resonance of the methyl carbon C-20.

(*Z*)-5-Ethylidene-2(5H)-furanone (**19**) was a major component of extracts of juvenile, but not of adult, foliage. This compound has proved to be an artefact produced by steam-distillation. The nature of the water-soluble precursor is under investigation.

Juvenile–adult chemical dimorphism

Juvenile *D. biforme* foliage extracts proved to be very different to extracts of adult foliage from the same plants. The significances of these differences were tested by Kruskal–Wallis one-way analysis of variance, as previously described [9]. The level of myrcene (**3**) was much lower in juvenile than in adult foliage, with median peak areas relative to the internal standard of 0.3 and 3.7 respectively (Table 1). The furanone (**19**) was one of the major components in juvenile extracts, but was barely detectable in adult extracts (Table 1). The totals of the sesquiterpene peak areas were lower in juvenile (median 2.6, relative to internal standard) than in adult extracts (median 8.8). These three differences were highly significant ($P < 0.01$). In four of the five juvenile foliage samples examined the major diterpene was 8α-isopimara-9(11),15-diene (**16**). By contrast, phyllocladene (**1**) predominated in all the adult samples. The relative levels of other diterpenes also changed (Table 2). This difference was not strictly significant ($P = 0.11$) but the anomalous result was for the largest plant, which had only a small amount of juvenile foliage. This sample was typically juvenile in other respects.

These contrasts between the chemical components of juvenile and adult *D. biforme* foliage are not surprising in view of the great morphological differences. However, only three previous references to juvenile–adult chemical dimorphism in gymnosperms have been found. Juvenile–adult foliage dimorphism is not met in all the gymnosperms, but it is characteristic of the genus *Dacrydium* and is also observed in many genera of the

large family Cupressaceae [18]. It was in *Juniperus scopulorum*, a member of this family, that Adams found significant differences in mono- and sesquiterpene levels between juvenile and adult foliage (this species does not contain diterpenes) [19]. Aplin and Cambie examined one sample of each type from *D. kirkii* F. Muell., and found lower phyllocladene (**1**), isophyllocladene (**2**) and kaurene (**11**) levels in juvenile leaves [7]. In our work on *D. cupressinum*, although we found significantly lower phyllocladene (**1**) levels in juvenile foliage [9], the great variations of diterpenes from tree to tree, irrespective of foliage type, complicated the situation.

The correlation between low levels of tetracyclic diterpenes and juvenile foliage form in *D. biforme* suggests a causal, rather than a casual, relationship when the following points are also considered:

1. The gibberellin plant growth hormones are biosynthesized from *ent*-kaurene (**20**) [20].
2. Gibberellin levels have been shown to be related to diterpene hydrocarbon levels in the foliage of *Cryptomeria japonica* D. Don (Taxodiaceae) [21].
3. Gibberellins affect many aspects of the lives of conifers [22]. Although changes in leaf form have not been recorded, flowering—obviously an adult attribute—was induced in numerous Cupressaceae by gibberellin treatment [22].

These observations lead us to the hypothesis that the change from juvenile to adult foliage forms in *Dacrydium* species is controlled by the production of gibberellins, whose biosynthesis in juvenile leaves is blocked at the stage of cyclization to form ring D of *ent*-kaurene (**20**). This is the one step in the proposed biosynthesis of *ent*-kaurene (**20**) which has not been modelled *in vitro*, and whose mechanism is in doubt [23, 24]. The necessity of enzymatic involvement in this step makes it a probable control point.

EXPERIMENTAL

General. Small scale extractions and GC analyses were performed as in ref. [9]. Additional quantitative data were obtained from isothermal GC analyses on a CW 20M capillary column. GC retention indices were measured as in ref. [11] and are given in Table 3. Specific rotations and NMR spectra were obtained as $CHCl_3$ and $CDCl_3$ solns respectively.

Sample collection. Dacrydium biforme adult foliage samples were collected by B. J. Gilbertson, New Zealand Forest Service, from Kaniere State Forest, grid reference 475228 (S57), altitude 280 m. Two collections were made, one in June, 1982, and the other a month later. Juvenile and adult foliage samples were collected by T. L. Dee and N. B. Perry from Mount Cargill, grid reference 200790 (S164), altitude 600 m, in October, 1983.

Table 3. Retention indices for compounds involved in this study*

Compound	Stationary phase	
	SE-30	CW 20M
Myrcene	985 (100°), 989 (130°)	1174 (130°)
Furanone (19)	994 (100°), 1002 (130°)	1748 (150°)
α-Longipinene	1359 (130°)	1524 (150°)
Longifolene	1409 (130°)	1624 (150°)
Caryophyllene	1420 (130°)	1639 (150°)
Germacrene D	1473 (130°)	1736 (150°)
Bicyclogermacrene	1488 (130°)	1757 (150°)
δ-Cadinene	1510 (130°)	1773 (150°)

*For diterpenes, see ref. [11].

Bulk extraction of adult foliage. Dacrydium biforme adult foliage (Tree 9, 174 g) was steam-distilled with concurrent extraction into hexane [25] for 48 hr. Solvent removal gave a crystalline residue (1.2 g). Recrystallization from EtOH gave phyllocladene (**1**; 0.7 g), mp 88–90° (lit. 98° [26]), $[\alpha]_D$ + 16° (lit. + 16° [26]).

Bulk extraction of juvenile foliage. Dacrydium biforme juvenile foliage (Tree 11, 127 g) was extracted as above. Evaporation of the hexane extract gave a green oil (1.4 g) which was chromatographed on a short alumina column. Elution with hexane gave a clear oil (0.36 g) which consisted largely of diterpenes. Elution with Et_2O gave a liquid (0.54 g) which, on silica gel prep. TLC (hexane–Et_2O, 1:1) gave (*Z*)-5-ethylidene-2(5H)-furanone (**19**) (0.055 g from 0.096 g); UV λ_{max} nm (log ε): 272 (4.1); IR ν_{max} cm^{-1}: 3160, 3120, 3080, 1870, 1790, 1690, 1340, 1240, 1135, 1010, 920, 865; ^1H NMR (90 MHz): δ1.97 (3H, *d*, *J* = 7.5 Hz, H-7), 5.34 (1H, *q*, *J* = 7.5 Hz, H-6), 6.11 (1H, *d*, *J* = 5.5 Hz, H-3), 7.33 (1H, *d*, *J* = 5.5 Hz, H-4); ^{13}C NMR (15.04 MHz): δ11.4 (*q*, $^1J_{CH}$ = 94 Hz, C-7), 111.6 (*dq*, $^1J_{CH}$ = 94 Hz, $^2J_{CH}$ = 7 Hz, C-6), 118.3 (*d*, $^1J_{CH}$ = 184 Hz, C-3), 143.3 (*d*, $^1J_{CH}$ = 178 Hz, C-4), 150.2 (*s*, C-4), 169.5 (*s*, C-2).

The hydrocarbon mixture (0.143 g), after prep. TLC on $AgNO_3$–silica gel (hexane–C_6H_6, 1:1) gave: (a) phyllocladene (**1**; 0.015 g), $[\alpha]_D$ + 17°; (b) sandaracopimaradiene (**12**; 0.013 g), $[\alpha]_D$ − 7° (lit. − 12° [26]); (c) 8α-isopimara-9(11),15-diene (**16**; 0.079 g), $[\alpha]_D$ − 15°; (Found: C, 88.1; H, 11.9. $C_{20}H_{32}$ requires C, 88.2; H, 11.8%); IR ν_{max} cm^{-1}: 3080, 1460, 990, 910; ^1H NMR (90 MHz): δ0.86 (3H, *s*), 0.90 (3H, *s*), 0.99 (3H, *s*), 1.08 (3H, *s*), 1.95 (2H, *m*, H-12), 2.19 (1H, *m*, H-8), 4.93 (1H, *m*, *J* = 1.4, 10.8 Hz, H-16*E*), 4.94 (1H, *m*, *J* = 1.4, 17.3 Hz, H-16*Z*), 5.33 (1H, *m*, $W_{h/2}$ = 6 Hz, H-11), 5.83 (1H, *m*, *J* = 10.8, 17.3 Hz, H-15); ^{13}C NMR (15.04 MHz): δ18.9 (*t*, C-2), 19.6 (*t*, C-6), 21.9 (*q*, C-19), 25.3 (*q*, C-20), 27.0 (*t*, C-7), 29.6 (*q*, C-17), 30.4 (*d*, C-8), 33.2 (*q*, C-18), 33.6 (*s*, C-4), 35.5 (*s*, C-13), 37.4 (*t*, C-1), 38.1 (*s*, C-10), 41.3 (*t*, C-12 or C-14), 42.6 (*t*, C-3), 43.9 (*t*, C-14 or C-12), 45.6 (*d*, C-5), 110.9 (*t*, C-16), 115.6 (*d*, C-11), 145.9 (*d*, C-15), 152.8 (*s*, C-9); EIMS (probe) 70 eV, *m/z* (rel. int.): 272 $[M]^+$ (55), 257 (100), 230 (25), 215 (13), 204 (11), 189 (16), 187 (16), 175 (16), 161 (30), 134 (30), 119 (39), 105 (52), 91 (60), 81 (41), 69 (48), 55 (52), 41 (70). Retention indices on CW 20M: 2168 (170°) and 2202 (190°); on SE-30: 1887 (170°) and 1910 (190°).

Isomerizations of 8α-isopimara-9(11),15-diene. (a) HCl was bubbled through a soln of 8α-isopimara-9(11),15-diene (**16**; 0.023 g) in $CHCl_3$ (5 ml) at room temp. for 45 min. The resulting soln was washed with satd aq. $NaHCO_3$ and H_2O, and evaporated to give isopimara-8(15)-diene (**13**; 0.023 g), $[\alpha]_D$ + 69° (lit. + 119° [13]); IR and ^1H NMR spectra as in ref. [13].

(b) A soln of 8α-isopimara-9(11),15-diene (**16**; 0.006 g) in HOAc (0.8 ml), H_2O (0.1 ml) and H_2SO_4 (0.1 ml) was kept at 56° for 9 hr. Dilution with H_2O and extraction into hexane gave a soln which was analysed by GC. Rimua-5(10),15-diene (**14**; 35%) and isopimara-8,15-diene (**13**; 13%) were identified by their GC retention indices on SE-30 and CW 20M capillary columns at 170 and 190° [11]. An additional component (22%) had GC retention indices on CW 20M of 2163, 170° and 2191, 190° and on SE-30 of 1929, 170° and 1949, 190°.

Cold solvent extraction of adult foliage. Freshly ground *D. biforme* adult foliage (Tree 11, 500 g) was stirred at room temp. with hexane (2 l.) for 20 hr. The extract was filtered and concd to 150 ml. Passage through a short column of alumina removed

polar materials and carotenes. A concd hexane soln of the non-polar material was kept in a refrigerator overnight, during which time much of the phyllocladene crystallized out. A ^{13}C NMR spectrum of the mother liquors after the removal of solvent showed peaks consistent with the presence of myrcene (**3**), δ-cadinene (**9**), germacrene D (**7**) and bicyclogermacrene (**8**) [27]. These substances were all isolated by CC on $AgNO_3$–silica gel and had IR, ^{1}H NMR and ^{13}C NMR spectra consistent with literature data [16, 27–31]. δ-Cadinene had $[\alpha]_D$ 42° (lit. 92°), germacrene D had $[\alpha]_D$ −230° (lit. −240°) and bicyclogermacrene had $[\alpha]_D$ 73° (lit. 61°).

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