

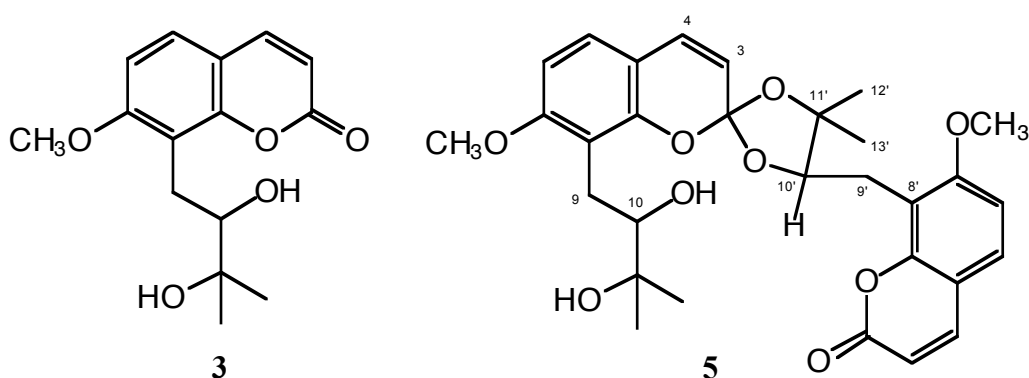
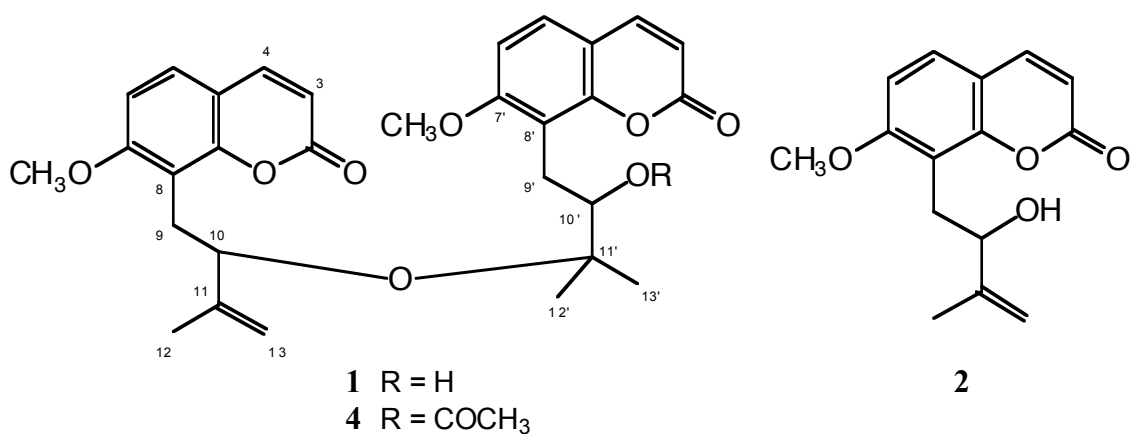
## TWO NEW BICOUMARINS FROM THE LEAVES OF *MURRAYA EXOTICA*

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**Abstract** - Two new bicoumarins, named murradimerin A (**1**) and murramarin B (**5**) were isolated from the leaves of *Murraya exotica* (Rutaceae) collected in Ishigaki-island Japan. Their structures were elucidated on the basis of spectroscopic data especially using 2D NMR spectrum. Naturally occurring bicoumarin connected two coumarin moieties by orthoester structure as in murramarin (**5**) is unusual type and murramarin B (**5**) is the first example not possessing furanocoumarin moiety.

We have previously reported that the leaves of *Murraya exotica* contains many coumarins.<sup>1</sup> Although *Murraya* species have been used as folk medicine in India, Australia and South Africa,<sup>2</sup> the plant of *M. exotica* has been considered to be a synonym of *M. paniculata*. However, investigations led to the proposal that this species is to be re-instated as a distinct taxon.<sup>3</sup> Under these circumstances, studies of the constituents of this plant are interesting from the point of chemotaxonomy. The present paper describes the isolation and characterization of two new bicoumarins, murradimerin A and murramarin B. **Murradimerin A (1)** was obtained as a colorless oil,  $[\alpha]_D - 38.0^\circ$  (MeOH). The IR (1723, 1609  $\text{cm}^{-1}$ ) and UV [220 (sh), 248, 258 and 322 nm] spectral data showed the presence of 7-oxygenated coumarin nucleus.<sup>4</sup> The molecular formula  $\text{C}_{30}\text{H}_{32}\text{O}_8$  was determined by HR-MS spectrum which showed the molecular ion at  $m/z$  520.2076. The <sup>1</sup>H-NMR spectrum showed four pairs of AB type aromatic proton



signals [ $\delta$  7.61, 6.21 (each 1H, d,  $J = 9.5$  Hz), 7.60, 6.21 (each 1H, d,  $J = 9.5$  Hz), 7.32, 6.84 (each 1H, d,  $J = 8.8$  Hz), 7.30, 6.86 (each 1H, d,  $J = 8.4$  Hz)] and two methoxy groups [ $\delta$  3.93, 3.92 (each 3H, s)], suggesting the presence of two 7-methoxy-8-substituted coumarin skeletons. Other signals at  $\delta$  1.90 (3H, s), 4.78, 4.71 (each 1H, s) and 1.12, 0.85 (each 3H, s) showed the presence of isopropenyl group and geminal dimethyl groups, respectively. Two vicinal methylene and methine proton signals were observed at  $\delta$  3.14 (1H, dd,  $J = 8.4, 13.2$  Hz), 3.02 (1H, dd,  $J = 5.5, 13.2$  Hz), 4.35 (1H, dd,  $J = 5.5, 8.4$  Hz), 2.93 (1H, dd,  $J = 10.3, 13.6$  Hz), 2.73 (1H, dd,  $J = 2.9, 13.6$  Hz) and 3.58 (1H, dd,  $J = 2.9, 10.3$  Hz). The above data suggested that murradimerin A has a dimeric structure linked by ether bridge between hydroxyl groups of auraptanol (**2**)<sup>5</sup> and meranzin hydrate (**3**)<sup>6</sup>. Treatment of **1** with acetic anhydride/pyridine afforded the monoacetate (**4**), suggesting the presence of hydroxyl group in **1**. The above result showed the location of the linkage between C-10 of auraptanol and C-11' of meranzin hydrate. In order to establish the linking position and to assign the structure unambiguously, a series of NMR spectral experiments including H-H COSY, HMQC and HMBC was performed. In the HMBC spectrum (Figure 1), H-10 ( $\delta$  4.35) showed <sup>2</sup> $J$  and <sup>3</sup> $J$  correlations with four carbon resonances at  $\delta$  29.4 (C-9), 17.7 (C-12), 110.7 (C-13) and 78.7 (C-11'). The connectivities were also observed between H-9 ( $\delta$  3.02, 3.14)/ C-7, C-8, C-8a, C-10 and C-11; H-13 ( $\delta$  4.78, 4.71)/ C-12 and C-10; 13'-Me ( $\delta$  1.12)/ C-11' and C-10'; H-9'

( $\delta$  2.93, 2.73)/ C-7', C-8' and C-8'a. Due to these results, the linkage between C-10 of auraptenol and C-11' of meranzin hydrate was established. Though we tried to apply the Mosher method,<sup>7</sup> the acylation of hydroxyl group of **1** failed, probably due to steric hindrance from its surroundings, so the relative configuration of the chiral centers remains to be determined.

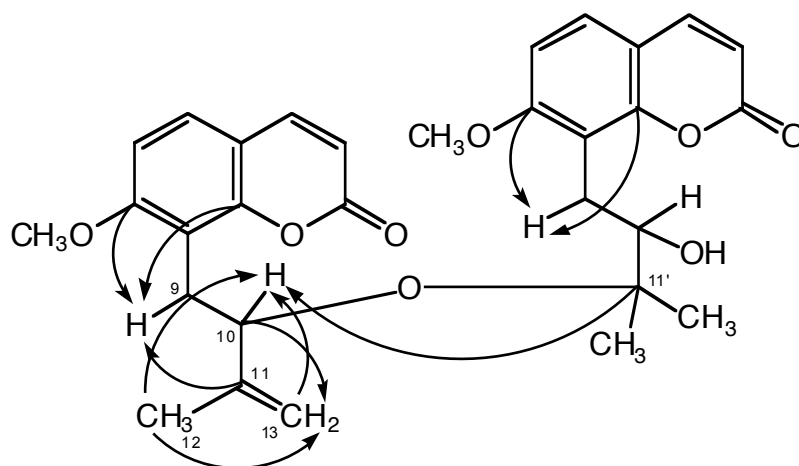


Figure 1 Key  $^3J_{CH}$  Long-Range Correlations in the HMBC Spectrum of **1**

**Murramarin B (5)** was isolated as a colorless oil,  $[\alpha]_D -57.1^\circ$  (MeOH). The FAB-MS of **5** showed a pseudomolecular ion peak at  $m/z$  539  $[M+H]^+$  and the molecular formula  $C_{30}H_{34}O_9$  was established by HR-FAB-MS spectrum. The IR (1723, 1610  $cm^{-1}$ ) and UV [240 (sh), 258 (sh), 274, 306, 322 nm] spectra suggested the presence of 7-oxygenated coumarin skeleton.<sup>4</sup> The  $^1H$ -NMR spectrum showed four C-methyl ( $\delta$  1.48, 1.39, 1.21 and 1.18), two O-methyl ( $\delta$  3.94 and 3.81), two methylene [ $\delta$  3.31 (1H, dd,  $J = 9.2, 13.9$  Hz), 2.96 (1H, dd,  $J = 4.0, 13.9$  Hz), 2.95 (1H, d,  $J = 13.6$  Hz), 2.73 (1H, dd,  $J = 9.5, 13.6$  Hz)], two oxy-methine [ $\delta$  4.61 (1H, dd,  $J = 4.0, 9.2$  Hz), 3.47 (1H, d,  $J = 9.5$  Hz)] and four pairs of olefinic proton [ $\delta$  7.60, 6.24 (each 1H, d,  $J = 9.5$  Hz), 7.33, 6.85 (each 1H, d,  $J = 8.4$  Hz), 6.98, 6.49 (each 1H, d,  $J = 8.4$  Hz), 6.70, 5.67 (each 1H, d,  $J = 9.5$  Hz)] signals. In the NOE experiments, irradiation of the methoxy signals at  $\delta$  3.94 and 3.81 induced 15 % and 14% increments of the signal at  $\delta$  6.85 (H-6') and 6.49 (H-6), respectively. Although these data were the case of the presence of two 7-methoxy-8-substituted coumarin moieties, the  $^{13}C$ -NMR spectrum showed the presence of only one carbonyl signal at  $\delta$  161.0 and unusual one carbon signal linked three oxygen atoms at  $\delta$  117.1, indicating that one of two lactone carbonyls in compound (**5**) was replaced by the orthoester structure as observed in the case of known spirobicoumarins; rivulobirins B, C<sup>8</sup> and paradisin C.<sup>9</sup> The C-H correlation in the HMBC spectrum (Figure 2) between  $\delta$  5.67 (H-3) / 117.1 (C-2) and NOE correlation between H-3/ 13'-Me ( $\delta$  1.39) suggested the presence of an orthoester structure in **5**. The relative configurations of the C-2 and

C-10' in **5** were confirmed by the analysis of its NOESY spectrum (Figure 3).

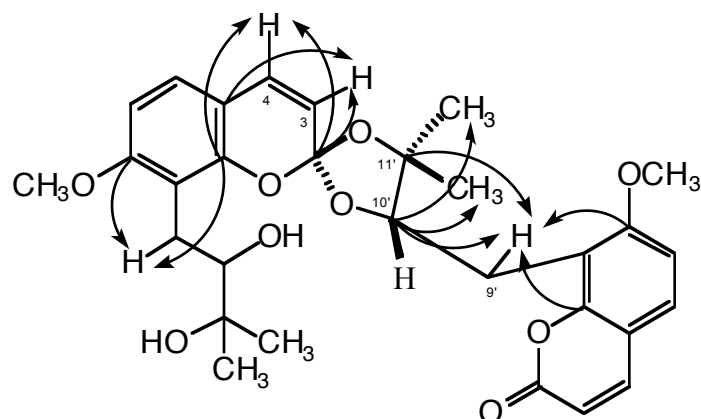


Figure 2 Key CH Long-Range Correlations in the HMBC Spectrum of **5**

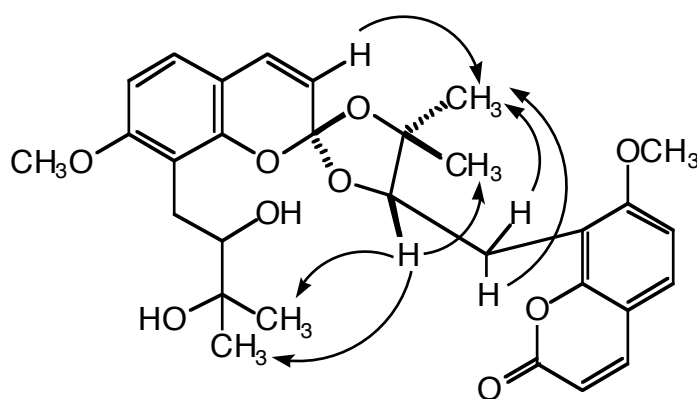


Figure 3 Key NOESY's of **5**

The whole structure of **5** was confirmed by the HMBC spectrum (Figure 2) and it was clarified to be composed of two meranzin hydrate moieties, the absolute configuration of the chiral centers remains to be determined. Spirobicoumarins whose one lactone carbonyl group is bounded to two hydroxyl groups of another coumarin moiety forming orthoester structure, have been isolated from *Pleurospermum rivulorum* (Umbelliferae)<sup>8</sup> and *Citrus paradisi* (Rutaceae).<sup>9</sup> All known spirobicoumarins were composed with furanocoumarin, while murramarin B is the first example which contains no furanocoumarin moiety.

## EXPERIMENTAL

<sup>1</sup>H- and <sup>13</sup>C-NMR, NOE, HMQC and HMBC ( $J = 8$  Hz) spectra were recorded on JNM A-400, A-600, and/or ECP-500 (JEOL) spectrometers in CDCl<sub>3</sub>. Chemical shifts are shown in  $\delta$  values (ppm) with tetramethylsilane (TMS) as an internal reference. UV spectra were recorded on a UVIDEC-610C double-beam spectrophotometer (JASCO) in MeOH. IR spectra on an IR-230 (JASCO) in CHCl<sub>3</sub>, and optical rotations on a DIP-370 (JASCO) in MeOH at 25°C. MS spectrum were taken with a HX-110 (JEOL) or

JMS-700 (JEOL) spectrometer having a direct inlet system. Preparative TLC was carried out on Kieselgel 60 F<sub>254</sub> (Merck).

**Extraction and Isolation** The leaves of *Murraya exotica* (Rutaceae) was collected in Ishigaki Island. A voucher specimen was deposited in the laboratory of Meijo University. The air-dried plant (1.0 kg) was extracted with acetone (3×6L) at rt for 7 days each time. The extract (136 g) was chromatographed on silica gel (1 kg) with successive elution with toluene, AcOEt:toluene and acetone. The acetone eluate was submitted to centrifugal chromatography eluted with CHCl<sub>3</sub> containing increasing amounts of acetone (CHCl<sub>3</sub>-acetone, 100:0 - 0:100). The CHCl<sub>3</sub> eluate (11.3 g) was submitted to repeated PTLC [solvent: acetone – hexane (4:6), acetone – CHCl<sub>3</sub> (2:8), AcOEt – hexane (9:1), AcOEt – benzene (7:3), isoPr ether] to give murradimerin A (**1**) (6.1 mg) and murradimarin B (**5**) (15.1 mg).

**Murradimerin A (1)**: Colorless oil,  $[\alpha]_D^{25} -38.0^\circ$  ( $c = 0.108$ , MeOH); HR-MS  $m/z$ : 520.2076 ( $M^+$ , found), 520.2095 (calcd for C<sub>30</sub>H<sub>32</sub>O<sub>8</sub>); EI-MS  $m/z$ : 520 ( $M^+$ ), 462, 261, 243, 190, 189; UV  $\lambda_{max}$  (MeOH, nm): 208, 220 (sh), 248, 258, 322; IR (CHCl<sub>3</sub>,  $\nu$  cm<sup>-1</sup>): 3528, 1723, 1609; <sup>1</sup>H-NMR (CDCl<sub>3</sub>,  $\delta$ ): 7.61 (1H, d,  $J = 9.5$  Hz, H-4'), 7.60 (1H, d,  $J = 9.5$  Hz, H-4), 7.32 (1H, d,  $J = 8.8$  Hz, H-5), 7.30 (1H, d,  $J = 8.4$  Hz, H-5'), 6.86 (1H, d,  $J = 8.4$  Hz, H-6'), 6.84 (1H, d,  $J = 8.8$  Hz, H-6), 6.21 (2H, d,  $J = 9.5$  Hz, H-3 and H-3'), 4.78 (1H, s, H-13), 4.71 (1H, s, H-13), 4.35 (1H, dd,  $J = 5.5, 8.4$  Hz, H-10), 3.93 (3H, s, 7'-OMe), 3.92 (3H, s, 7-OMe), 3.58 (1H, dd,  $J = 2.9, 10.3$  Hz, H-10'), 3.14 (1H, dd,  $J = 8.4, 13.2$  Hz, H-9), 3.02 (1H, dd,  $J = 5.5, 13.2$  Hz, H-9), 2.93 (1H, dd,  $J = 10.3, 13.6$  Hz, H-9'), 2.73 (1H, dd,  $J = 2.9, 13.6$  Hz, H-9'), 1.90 (3H, s, 12-Me), 1.12 (3H, s, 13'-Me), 0.85 (3H, s, 12'-Me). <sup>13</sup>C-NMR (CDCl<sub>3</sub>,  $\delta$ ): 161.3 (s, C-7), 161.1 (s, C-7'), 160.9 (s, C-2), 160.8 (s, C-2'), 153.6 (s, C-8a), 153.4 (s, C-8'a), 148.2 (s, C-11), 143.797 (d, C-4), 143.765 (d, C-4'), 126.7 (d, C-5), 126.5 (d, C-5'), 116.5 (s, C-8), 115.7 (s, C-8'), 113.0 (s, C-4a), 112.94 (d, C-3), 112.92 (d, C-3'), 112.7 (s, C-4), 110.7 (t, C-13), 107.4 (d, C-6), 107.3 (d, C-6'), 78.7 (s, C-11'), 77.8 (d, C-10'), 74.7 (d, C-10), 56.2 (q, 7-OMe), 56.1 (q, 7'-OMe), 29.4 (t, C-9), 24.7 (t, C-9'), 22.6 (q, 13'-Me), 19.7 (q, 12'-Me), 17.7 (q, 12-Me).

**Murradimerin A acetate (4)**: A pyridine (2 mL) solution of murradimerin A (**1**) (7.1 mg) and acetic anhydride (2 mL) was left at rt overnight, and then evaporated to dryness. The residue was purified by PTLC [solvent: acetone:CHCl<sub>3</sub> (2:8)] to afford murradimerin A acetate (**4**) (3.2 mg) as a colorless oil. **4**: EI-MS  $m/z$ : 562 [ $M$ ]<sup>+</sup>, 303, 261, 243, 190, 189 (base peak); IR (CHCl<sub>3</sub>,  $\nu$  cm<sup>-1</sup>): 1725, 1609; <sup>1</sup>H-NMR (CDCl<sub>3</sub>,  $\delta$ ): 7.60 (1H, d,  $J = 9.5$  Hz), 7.59 (1H, d,  $J = 9.5$  Hz), 7.30 (1H, d,  $J = 8.8$  Hz), 7.29 (1H, d,  $J = 8.8$  Hz), 6.83 (1H, d,  $J = 8.8$  Hz), 6.80 (1H, d,  $J = 8.8$  Hz), 6.20 (2H, d,  $J = 9.5$  Hz), 5.01 (1H, d,  $J =$

2.2 Hz), 4.99 (1H, d,  $J = 2.2$  Hz), 4.61 (1H, d,  $J = 11.7$  Hz), 4.38 (1H, t,  $J = 7.3$  Hz), 3.93 (6H, s), 3.24 (1H, dd,  $J = 10.3, 13.9$  Hz), 3.12 (2H, dd,  $J = 3.7, 7.3$  Hz), 2.99 (1H, d,  $J = 11.7$  Hz), 1.92 (3H, s), 1.75 (3H, s), 1.17 (3H, s), 1.04 (3H, s).  $^{13}\text{C}$ -NMR ( $\text{CDCl}_3$ ,  $\delta$ ): 170.4 (s), 161.2 (s), 161.2 (s), 161.0 (s), 160.9 (s), 153.6 (s $\times$ 2), 148.0 (s), 143.8 (d), 143.7 (d), 126.9 (d), 126.6 (d), 115.7 (s), 115.5 (s), 112.9 (d), 112.7 (d), 112.7 (d), 112.6 (s), 110.5 (t), 107.2 (d), 107.1 (d), 78.6 (d), 77.4 (s), 74.9 (d), 56.1 (q), 55.9 (q), 29.7 (t), 29.3 (t), 22.9 (q), 22.7 (q), 21.6 (q), 17.2 (q).

**Murramarin B (5):** Colorless oil,  $[\alpha]_{\text{D}} - 57.1^\circ$  ( $c = 0.13$ , MeOH); HR-FAB-MS  $m/z$ : 539.2299 ( $[\text{M}+\text{H}]^+$ , found), 539.2279 (calcd for  $\text{C}_{30}\text{H}_{35}\text{O}_9$ ); EI-MS  $m/z$ : 386, 368, 256, 236, 190 (base peak); UV  $\lambda_{\text{max}}$  (MeOH, nm): 220 (sh), 240 (sh), 258 (sh), 274, 306, 322; IR ( $\text{CHCl}_3$ ,  $\nu \text{ cm}^{-1}$ ): 3568, 1723, 1610;  $^1\text{H}$ -NMR ( $\text{CDCl}_3$ ,  $\delta$ ): 7.60 (1H, d,  $J = 9.5$  Hz, H-3'), 7.33 (1H, d,  $J = 8.4$  Hz, H-5'), 6.98 (1H, d,  $J = 8.4$  Hz, H-5), 6.85 (1H, d,  $J = 8.4$  Hz, H-6'), 6.70 (1H, d,  $J = 9.5$  Hz, H-4), 6.49 (1H, d,  $J = 8.4$  Hz, H-6), 6.24 (1H, d,  $J = 9.5$  Hz, H-3'), 5.67 (1H, d,  $J = 9.5$  Hz, H-3), 4.61 (1H, dd,  $J = 4.0, 9.2$  Hz, H-10'), 3.94 (3H, s, 7'-OMe), 3.81 (3H, s, 7-OMe), 3.47 (1H, d,  $J = 9.5$  Hz, H-10), 3.31 (1H, dd,  $J = 9.2, 13.9$  Hz, H-9'), 2.96 (1H, dd,  $J = 4.0, 13.9$  Hz, H-9'), 2.95 (1H, d,  $J = 13.6$  Hz, H-9), 2.73 (1H, dd,  $J = 9.5, 13.6$  Hz, H-9), 2.71 (1H, br s, OH), 2.52 (1H, br s, OH), 1.48 (3H, s, 12'-Me), 1.39 (3H, s, 13'-Me), 1.21 (3H, s, 12-Me), 1.18 (3H, s, 13-Me); Differential NOE: irradiation at  $\delta$  3.94 (7-OMe) gave 15% enhancement of the signal at  $\delta$  6.85 (H-6'); irradiation at  $\delta$  3.81 (7'-OMe) gave 14% enhancement of the signal at  $\delta$  6.49 (H-6').  $^{13}\text{C}$ -NMR ( $\text{CDCl}_3$ ,  $\delta$ ): 161.0 (s, C-2'), 160.5 (s, C-7'), 158.4 (s, C-7), 153.4 (s, C-8'a), 150.6 (s, C-8a), 143.7 (d, C-4'), 128.4 (d, C-4), 127.3 (d, C-5'), 125.7 (d, C-5), 118.1 (d, C-3), 117.1 (s, C-2), 114.2 (s, C-4'a), 114.1 (s, C-4a), 113.2 (d, C-3'), 113.1 (s, C-8'), 113.0 (s, C-8), 107.4 (d, C-6'), 103.7 (d, C-6), 83.2 (s, C-11'), 82.7 (d, C-10'), 78.4 (d, C-10), 72.7 (s, C-11), 56.2 (q, 7'-OMe), 55.8 (q, 7-OMe), 26.4 (t, C-9), 25.7 (q, C-12'), 25.6 (q, C-13), 23.6 (q, C-12), 23.1 (t, C-9'), 22.7 (q, C-13').

## ACKNOWLEDGEMENTS

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