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Synthesis and biological activity of both enantiomers of kujigamberol isolated from 85-million-years-old Kuji amber

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ABSTRACT

The full-structure of a norlabdane terpenoid, kujigamberol (**1**) was determined by total synthesis. Key features of the total synthesis are (1) installation of isopentyl group through an *o*-lithiation of benzamide, (2) construction of tetralone by the RCM reaction, and (3) optical resolution of (±)-**1** using chromatographical separation of the corresponding camphanates. X-ray crystallographical analysis of *p*-bromobenzoate obtained from the more polar camphanate that was identical with a natural derivative, revealed natural kujigamberol to have an *S*-configuration. Both the natural enantiomer and its (*R*)-antipode showed the same inhibitory activity toward the mutant yeast and HL-60 cells, while simple analogs without alkyl groups at the C-8 and 9 positions of (±)-**1** had no such activity

Ambers are fossilized tree resins which are formed under favorable circumstances in soils and sediments, where the hardened resins can be preserved for up to hundreds of millions of years. Several kinds of origins and/or geological ages are reported in ambers such as Dominican (Dominican Republic), Mexican (Mexico), Baltic (Poland), Cedar Lake (Canada), New Jersey (USA), Spain (Spain), Lebanese (Lebanon) and Dolomites (Italy).[1,2] Among them, one of the oldest types is Kuji (Iwate Prefecture in Japan) amber whose geological age is dated at 85 million years. Recently, several papers have appeared dealing with chemical composition of amber.[3,4] However, there is no precedent for the study of compounds from any amber focused on their biological activity and activity guided fractionation. In our screening program for a new Ca^{2+}-signal transduction inhibitor by using the mutant yeast (*Saccharomyces cerevisiae*, *zds1*Δ *erg3*Δ *pdr1*Δ *pdr3*Δ),[5] we found the MeOH extracts of Kuji amber to be positive for the assay. Extensive studies using spectroscopic methods revealed that the active principle was a new norlabdanoid named kujigamberol (Fig. 1).[6] However, the absolute configuration has not been determined. Its occurrence in nature was also limited because of the rarity of the origin. To determine the absolute configuration of kujigamberol and evaluate the biological activity exactly, development of a method for preparation of both enantiomers of kujigamberol was required.

Described herein is the total synthesis of kujigamberol and its antipode, thus establishing the absolute configuration of the natural product. Their inhibitory activities are also discussed.

In the synthetic study of kujigamberol, introduction of isopentyl group into the aromatic ring at the later stage of total synthesis was estimated to be difficult. Hence, the synthetic strategy is designed as shown in Scheme 1. Both enantiomers would be obtained by an optical resolution of (±)-**1**. The quaternary carbon center of **1** would be constructed from a ketone at the C-4 position of tetralone **2**. C_1–C_2 bond[7] cleavage in **2** and changing the oxidation level can revert it back to γ-lactone **3**. This could be synthesized from **4** via a chain elongation including Stille coupling.[8] These retrosynthetic analyses allowed us to select benzamide **5**[9] as the starting material.

Synthesis of (±)-**1** began with installation of isopentyl group on an aromatic ring in **5** (Scheme 2). Since *o*-lithiation[10] of **5** followed by alkylation with an alkyl halide such as prenyl bromide failed, the aryl lithium was trapped by DMF. The aldehyde **6** thus obtained was treated with the phosphorane prepared from isobutylphosphonium bromide and *n*-BuLi in THF to give **7** after hydrogenation reaction. Demethylation of **7** provided **8**, which was transformed into the corresponding triflate **4**. Stille coupling[11] of **4** with tributyl(vinyl)tin was effected by treatment with dichlorobis(triphenylphosphine)palladium in the presence of lithium chloride and triphenylphosphine to give **9** in high yield. Hydride reduction of **9** did not proceed cleanly or gave the 1,6-reduction product predominantly. Direct hydrolysis of the amide carbonyl under acidic or basic conditions was also found to be difficult. After

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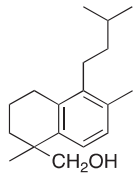
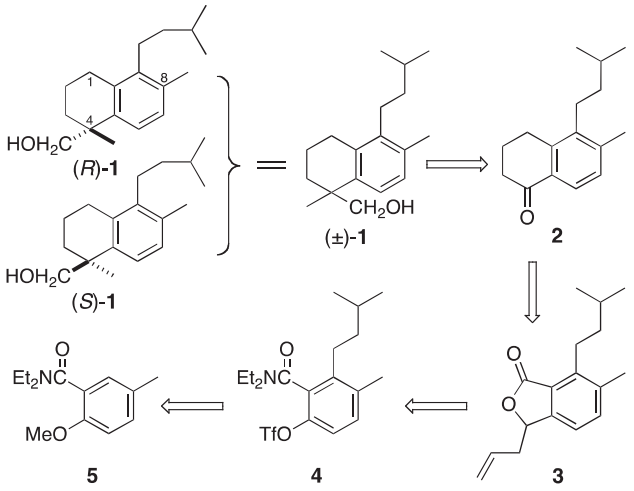
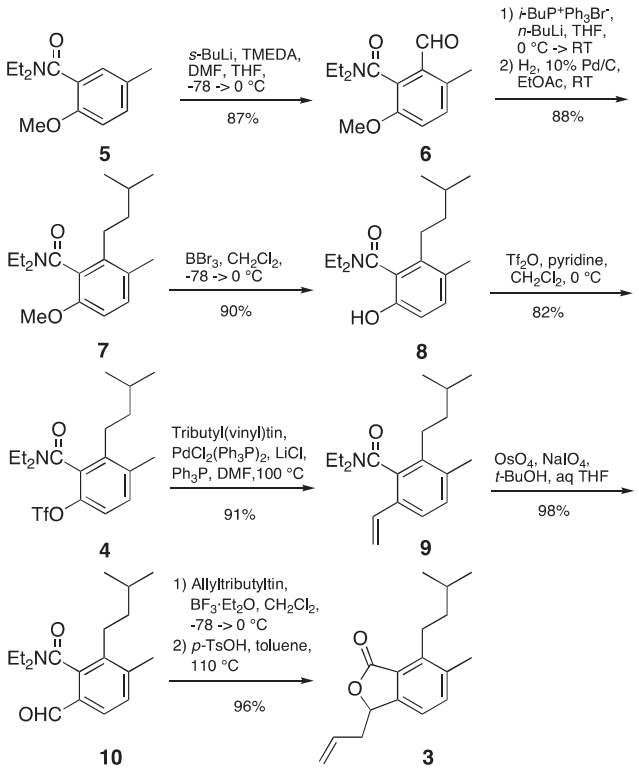


Figure 1. Planar structure of kujigamberol.



Scheme 1. Synthetic plan of kujigamberol.



Scheme 2. Synthesis of γ-lactone **3**.

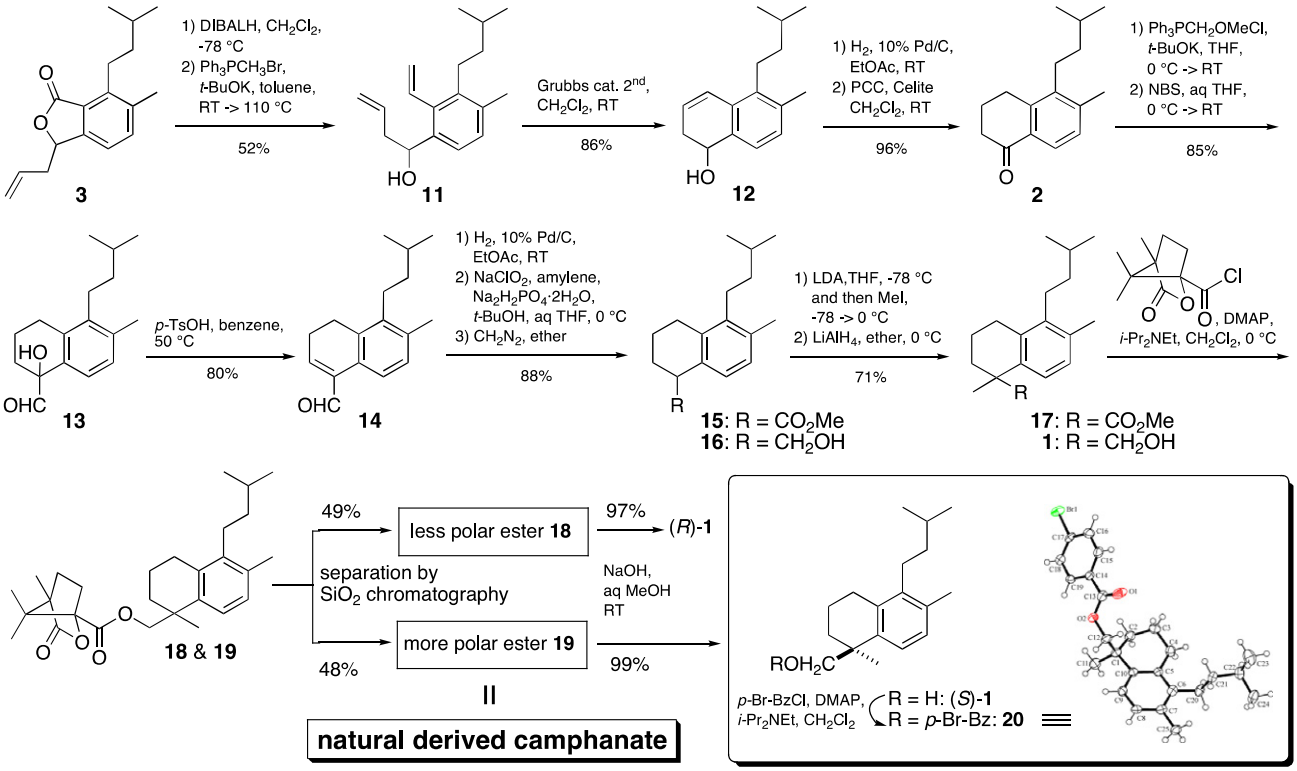
all, this transformation was attained by the neighboring group participation of an internal hydroxyl group as follows. Thus, **9** was initially oxidized by Lemieux–Johnson oxidation to give aldehyde **10**. Reaction of **10** with allyltributyltin[12] provided the corresponding alcohol, which was successively treated with *p*-TsOH to give rise to lactonization, giving γ-lactone **3**[13] in high yield.

For construction of the tetralone ring, **3** was reduced with DIBALH and the resulting hemiacetal reacted with methylenetriphenylphosphorane under forcing conditions, affording **11** (Scheme 3). Ring-closing metathesis of this with Grubbs cat. 2nd generation[14] proceeded nicely to give **12** in good yield. Hydrogenation of **12** followed by PCC oxidation provided tetralone **2**.[13] One carbon homologation[15] from **2** was performed by the action of methoxymethylene triphenylphosphorane to give the corresponding enol ether. Hydrolysis of the product under acidic conditions resulted in a low yield of a tetralin carbaldehyde derivative, while on treatment with NBS in aq THF, hydroxy aldehyde **13** was obtained in high yield. Since hydrogenolysis of **13** led to a considerable amount of **2**, **13** was subjected to dehydration reaction with *p*-TsOH to give α,β-unsaturated aldehyde **14**. This compound was, after hydrogenation, oxidized to provide the corresponding methyl ester **15**. The lithium enolate generated from the ester by the action of LDA reacted with iodomethane, and the resulting compound[18] was reduced with LAH, affording (±)-**1**.[19] The ^{1}H and ^{13}C NMR spectra of the synthetic sample were identical with those of the natural product.

For optical resolution of (±)-**1**, the racemic alcohol was acylated with (1*S*)-(−)-camphanic chloride to give a diastereomeric mixture of the corresponding camphanates **18** and **19**.[13] Each compound was separated by chromatography on silica gel with *n*-hexane–ethyl acetate. More polar isomer **19** was identical in all respects with an authentic sample derived from the natural product.[20] Hydrolysis of **19** gave (−)-**1**.[13] Its absolute configuration was determined to be *S* by X-ray crystallographical analysis[21] of the corresponding *p*-bromobenzoate **20**, establishing that the natural product has an *S*-configuration. Similarly, unnatural form, (+)-(*R*)-**1** was prepared.

Next we evaluated the biological activities[22,23] of the two enantiomers. Synthetic (*S*)-**1** showed a clear growth restored zone against the mutant yeast at 0.5–0.0156 μg/spot and cytotoxicity against human promyelocytic leukemia cell line HL60 (IC_{50} = 24.0 μM). These results were not distinguished from those of the natural product. Interestingly, the *R*-enantiomer and **16** also showed the same inhibitory activities. Encouraged by the above results, we examined the biological activities of simple analogs **21–23** without alkyl groups at the C-8 and 9 positions of **16**, **17**, or (±)-**1**, respectively (Fig. 2).[24] All analogs, however, had no inhibitory activity. The results suggest that the C_4 stereochemistry does not affect the inhibitory activity and that alkyl groups at the C-8 and/or 9 positions rather than substituents around the C-4 are important for inhibition potential.

In summary, the absolute configuration of kujigamberol was determined to be *S* by the total synthesis. Key features of our total synthesis are (1) installation of the isopentyl group on the aromatic ring, (2) construction of tetralone **2** by the RCM reaction, and (3) optical resolution of (±)-**1** using (1*S*)-(−)-camphanic chloride. Both natural enantiomer and its antipode showed the same inhibitory activity toward the mutant yeast and HL60 cells.



Scheme 3. Total synthesis of kujigamberol **1**.

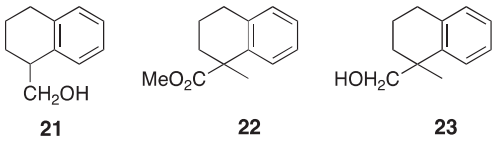


Figure 2. Simple analogs of kujigamberol.

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12. Treatment of **10** with allylmagnesium bromide furnished the corresponding homoallyic alcohol in a low yield.
13. *Spectral data of representative compounds:* Compound (*S*)-**1**: $[\alpha]_D^{26}$ −11.2 (*c* = 0.23, methanol) {lit.[6] $[\alpha]_D^{29}$ −8.5 (*c* = 1.0, methanol)}; ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.07 (1H, d, *J* = 8.2 Hz), 7.00 (1H, br d, *J* = 8.2 Hz), 3.78 (1H, d, *J* = 11.0 Hz), 3.51 (1H, d, *J* = 11.0 Hz), 2.76 (1H, ddd, *J* = 16.5, 5.9, 5.9 Hz), 2.68 (1H, ddd, *J* = 16.5, 8.2, 5.5 Hz), 2.57 (2H, t, *J* = 8.8 Hz), 2.28 (3H, s), 1.97 (1H, ddd, *J* = 13.0, 10.0, 3.1 Hz), 1.88 (1H, m), 1.79 (1H, m), 1.68 (1H, m), 1.52 (1H, m), 1.36–1.23 (2H, m), 1.24 (3H, s), 0.98 (6H, d, *J* = 6.9 Hz); ^{13}C NMR (150 MHz, $CDCl_3$): δ 139.8, 138.8, 136.1, 133.6, 128.0, 123.9, 71.9, 39.5, 37.9, 32.9, 29.0, 27.4, 27.2, 26.9, 22.45, 22.44, 19.6, 19.5; HRMS (ESI^+) calcd for $C_{18}H_{28}ONa$ $[M+Na]^+$ 283.2038, found 283.2044.
Compound **2**: ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.84 (1H, d, *J* = 8.0 Hz), 7.11 (1H, d, *J* = 8.0 Hz), 2.93 (2H, t *J* = 6.1 Hz), 2.65–2.59 (4H, m), 2.36 (3H, s), 2.14–2.11 (2H, m), 1.70 (1H, m), 1.35–1.30 (2H, m), 0.99 (6H, d, *J* = 6.9 Hz); ^{13}C NMR (125 MHz, $CDCl_3$): δ 198.7, 142.1, 139.3, 131.2, 128.6, 124.8, 38.5, 38.0, 28.7, 27.1, 26.2, 22.9, 22.4, 20.4; HRMS (EI^+) calcd for $C_{16}H_{22}O$ $[M]^+$ 230.1671, found 230.1677.
Compound **3**: ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.39 (1H, d, *J* = 7.8 Hz), 7.13 (1H, d, *J* = 7.8 Hz), 5.76 (1H, m), 5.36 (1H, t, *J* = 5.9 Hz), 5.17 (1H, ddd, *J* = 17.1, 2.9, 1.5 Hz), 5.13 (1H, ddd, *J* = 10.3, 2.9, 1.2 Hz), 3.13–3.09 (2H, m), 2.69 (1H, dt, *J* = 14.5, 6.6 Hz), 2.59 (1H, dt, *J* = 14.5, 6.6 Hz), 2.38 (3H, s), 1.74 (1H, m), 1.40–1.37 (2H, m), 0.99 (6H, d, *J* = 6.6 Hz); ^{13}C NMR (125 MHz, $CDCl_3$): δ 170.3, 147.9, 143.4, 137.3, 135.8, 131.6, 123.2, 119.3, 118.7, 78.5, 39.1, 39.0, 28.7, 25.3, 22.43, 22.40, 18.5; HRMS (EI^+) calcd for $C_{17}H_{22}O_2$ $[M]^+$ 258.1620, found 258.1615.
Compound **18**: $[\alpha]_D^{24}$ +14.5 (*c* = 1.02, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.06 (2H, br d, *J* = 7.7 Hz), 6.97 (2H, br d, *J* = 7.7 Hz), 4.47 (1H, d, *J* = 11.0 Hz), 4.10 (1H, d, *J* = 11.0 Hz), 2.75 (1H, dt, *J* = 17.0, 6.0 Hz), 2.68 (1H, ddd, *J* = 17.0, 6.9, 6.0 Hz), 2.55 (2H, m), 2.28 (1H, m), 2.26 (3H, s), 1.94 (1H, m), 1.91–1.85 (2H, m), 1.80 (1H, m), 1.69–1.58 (3H, m), 1.57 (3H, s), 1.33 (3H, s), 1.30–1.25 (3H, m), 1.08 (3H, s), 0.98 (6H, d, *J* = 6.9 Hz). 0.94 (3H, s), 0.77 (3H, s); ^{13}C NMR (150 MHz, $CDCl_3$): δ 178.2, 167.3, 139.6, 138.0, 135.3, 133.9, 127.9, 124.3, 91.3, 73.0, 54.8, 53.9, 37.9, 37.7, 33.4, 30.5, 28.94, 28.88, 27.3, 27.0, 26.9, 22.4, 19.6, 19.3, 16.7, 16.5, 9.7; HRMS (ESI^+) calcd for $C_{28}H_{40}O_4Na$ $[M+Na]^+$ 463.2824, found 463.2837.
Compound **19**: $[\alpha]_D^{24}$ −30.3 (*c* = 1.13, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.06 (2H, br d, *J* = 7.8 Hz), 6.97 (2H, br d, *J* = 7.8 Hz), 4.44 (1H, d, *J* = 11.0 Hz), 4.12 (1H, d, *J* = 11.0 Hz), 2.77 (1H, dt, *J* = 17.0, 6.0 Hz), 2.68 (1H, ddd, *J* = 17.0, 7.3, 6.0 Hz), 2.55 (2H, m), 2.32 (1H, m), 2.26 (3H, s), 1.98 (1H, m), 1.93–1.75 (3H, m), 1.70–1.55 (3H, m), 1.32 (3H, s), 1.30–1.25 (3H, m), 1.07 (3H, s), 0.98 (6H, d, *J* = 6.4 Hz). 0.81 (3H, s), 0.80 (3H, s); ^{13}CNMR (150 MHz, $CDCl_3$): δ 178.1, 167.3, 139.6, 138.0, 135.2, 133.9, 128.0, 124.2, 91.3, 73.1, 54.7, 53.9, 37.9, 37.7, 33.4,

30.6, 30.3, 29.0, 28.9, 27.3, 27.1, 26.9, 22.4, 19.6, 19.3, 16.5, 16.4, 9.7; HRMS (ESI^+) calcd for $C_{28}H_{40}O_4Na$ $[M+Na]^+$ 463.2824, found 463.2818.
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18. This compound was shown to be an inseparable mixture of **17** and recovered **15** by ^1H NMR analysis. From a practical point of view, separation of the mixture after the following reduction was found to be more efficient.
19. Along with **1**, alcohol **16** derived from unreacted **15** was also isolated in 11% yield.
20. It was revealed that the natural product contained no antipode by TLC and ^1H NMR analyses (500 MHz) of the crude products after esterification with (1*S*)-(−)-camphanic chloride.
21. The carbon numbering system is different from that employed in Scheme 1. The detailed crystallographic data for **20** have been deposited with the Cambridge Crystallographic Data Centre: Deposition code CCDC 852847. A copy of the data can be obtained free of charge via http://www.ccdc.cam.ac.uk/products/csd/request.
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