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Synthesis of DL-standishinal and its related compounds for the studies on structure–activity relationship of inhibitory activity against aromatase

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Abstract—DL-Standishinal (**1**), an aromatase inhibitor isolated from *Thuja standishii*, was synthesized in 15 steps from *p*-formylanisole via aldol reaction of 12-hydroxy-6,7-secoabieta-8,11,13-trien-6,7-dial (**2**). In the present study, we found that the aldol condensation of **2** proceeded in excellent yield with the protonic catalyst such as *d*-camphorsulfonic acid in CH_2Cl_2. Moreover, structure-activity relationship of **1** and its related compounds was studied and it was revealed that the isomers having *cis*-configuration on the A/B-ring generally exhibited more potent inhibitory activities against aromatase than those with *trans*-configuration.

1. Introduction

Breast cancer is an aromatase-dependent cancer and has been the major cause of death of women in recent times.[1] Since the aromatase catalyzes the conversion of androgens into estrogens, inhibitors against the enzyme are considered to be valuable as therapeutic agents in the treatment of breast cancer. Both steroidal and non-steroidal compounds are known as aromatase inhibitors, that is, exemestane[2] is an example of the former, while fadrozole,[3] anastrozole,[4] and letrozole[5] released as second and third generations of marketed medicines belong to the latter. Recent reports demonstrate that the third generation of aromatase inhibitors is more effective than tamoxifen in postmenopausal breast cancer patients[6], so aromatase inhibitors are mainly used today for the treatment of advanced breast cancer of postmenopausal women. While many kinds of inhibitors have already been reported,[7–18] one of the authors found that a family of diterpenes, such as standishinal (**1**) isolated from *Thuja standishii* (Gord.) Carr. (*Cupressaceae*), is another example of the inhibitors.[19–21] Standishinal (**1**) is not only a promising lead candidate of anti-breast cancer agents but it also has an unusual 6(7 → 11)*abeo*-abietane skeleton. Although several synthetic approaches to natural products having the *abeo*-abietane skeleton such as dichroanals and taiwanquinolines were reported in recent times,[22–26] their methods were not applicable to the synthesis of **1** with the A/B *trans*-configuration. On the other hand, Tanaka succeeded in the chemical conversion from 12-hydroxy-6,7-secoabieta-8,11,13-trien-6,7-dial (**2**), another component of the same plant source, to **1** by Lewis acid (BF_3·Et_2O)-catalyzed aldol reaction (Fig. 1).[19] On the basis of these backgrounds, we would like to report the first total synthesis of DL-**1** by using the acid-catalyzed aldol reaction of **2** as a key step. Moreover, structure-activity relationship of **1** and its derivatives obtained during the synthetic study of **1** would also be discussed.

Keywords: Standishinal; Aromatase; Inhibitor; *abeo*-abietane.
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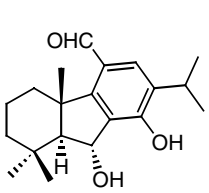
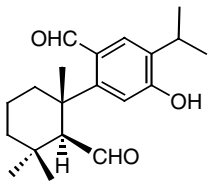
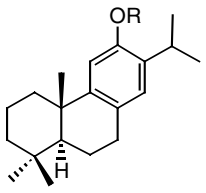


(-)-standishinal (**1**)



12-hydroxy-6,7-secoabieta-8,11,13-triene-6,7-dial (**2**)

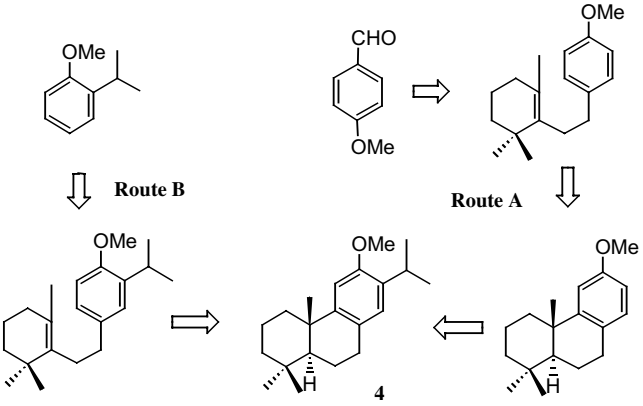


ferruginol (**3**): R = H
O-methyl ferruginol (**4**): R = Me

Figure 1. Structures of standishinal (**1**) and its related compounds.

2. Results and discussion

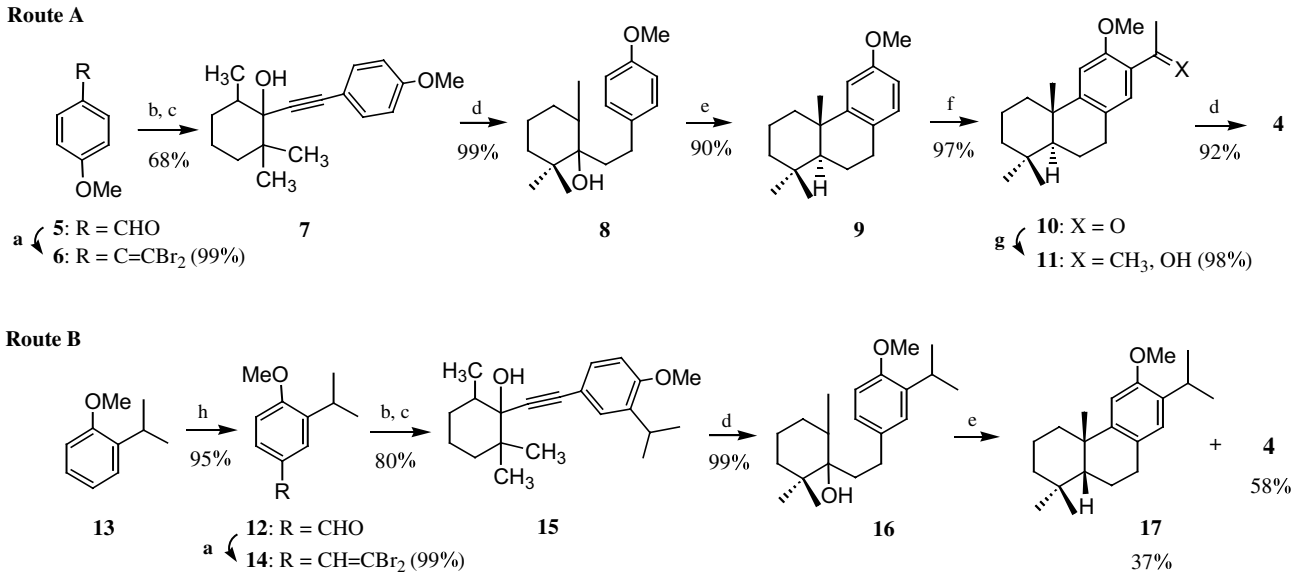
From a viewpoint of biosynthesis, the dialdehyde (**2**) could be derived from ferruginol (**3**) by the oxidative cleavage of the C–C bond between C-6 and C-7. Thus, ferruginol (**3**) or ferruginyl methyl ether (**4**) was chosen as an intermediate in the synthesis of **1**. Synthesis of **3** has been reported by several groups to date.[27–32] Ramana's group developed a domino reaction of anisole with (2,6,6-trimethylcyclohex-1-enyl)acetic acid, which was easily prepared from citral, to attain a facile synthesis of **3**.[28] Ogasawara and his colleagues also found a novel single-step reaction for constructing hexahydrophenanthrene, which was facilely converted to (+)-**3**.[29] King's and Tada's groups succeeded in short-step total synthesis of **3** (10 steps), however, the overall yields were lower than 10%.[30,31] Tada and his colleagues, furthermore, applied the method to the synthesis of (+)-**3** using optical resolution by deriving to esters with (*R*)-binaphthol.[31] On the other hand, Pan and his colleague also reported the synthesis of optically active ferruginyl methyl ether (**4**) in seven steps; however, it required a chiral aldehyde which had to be prepared by taking four steps from a commercially available substrate.[32] Thus, we started the synthesis of **1** with the preparation of ferruginyl methyl ether (**4**) based on the King's method[30] since the King's synthetic route can be easily modified for the asymmetric synthesis of **3** by taking advantage of our recent study on the synthesis of (−)-podocarpic acid using lipase-catalyzed reaction.[33] Two routes were herein applicable to prepare **4**, that is, one route advanced annulation of the B-ring followed by introduction of isopropyl group into the C-ring (Route A, King's method), and the other adopted B-ring's cyclization of the substrate having isopropyl group on the C-ring (Route B, modified King's method) (Scheme 1).



Scheme 1. Synthetic strategy of *O*-methyl ferruginyl ether (**4**).

First, *p*-methoxybenzaldehyde (**5**) was derived to dibromoolefin (**6**) by Corey–Fuchs's method[34] and **6** was converted to lithium acetylide which was treated with 2,2,6-trimethylcyclohexanone to afford propargyl alcohol (**7**) according to the King's method (Route A). The triple bond of **7** was reduced by catalytic hydrogenation on Pd–C, and obtained tertiary alcohol (**8**) was cyclized with Eaton's reagent (P_2O_5 in CH_3SO_3H) to afford tricyclic compound (**9**). Friedel–Crafts acylation of **9** with acetyl chloride in the presence of aluminum chloride gave **10**, which was reacted with methylmagnesium bromide to give **11**. Hydrogenolysis of **11** afforded **4**[32] in excellent yield. Next, Route B was attempted, that is, 3-isopropyl-4-methoxybenzaldehyde (**12**) prepared from *o*-isopropylanisole (**13**) with dichloromethoxymethane and tin(IV) chloride was derived to dibromoolefin (**14**), which was successively treated with *n*-butyllithium and 2,2,6-trimethylcyclohexanone to afford propargyl alcohol (**15**). The triple bond of **15** was reduced by hydrogenation to afford tertiary alcohol (**16**). Showing a good contrast to the acid-catalyzed cyclization of **8**, treatment of **16** with Eaton's reagent afforded a mixture of **4** and its epimer at C-5 (**17**) (Scheme 2). The structure of **17** was secured mainly by the δ value of the signal assigned to the C4-α methyl group, which should appear around at δ 0.4 ppm in the ^{1}H NMR spectra.[32,35]

The differences in the stereochemistry of the A/B ring on cyclization **8** and **16** could be explained by the difference in the rate of protonation on the methoxy group. Namely, in the cyclization of **8**, protonation on the methoxy group occurred faster than successive protonation on the double bond produced by dehydration of the hydroxyl group at C-5, and the latter protonation generated tertiary carbocation. Once the carbocation was formed, intramolecular Friedel–Crafts alkylation proceeded at the meta-position of the methoxy group due to the decrease in the electron density of the aromatic ring. On the other hand, in the cyclization of **16**, protonation hardly occurred on the methoxyl group due to the bulkiness of the neighboring *iso*-propyl group, therefore, the para-position of the methoxy group reacted with the tertiary carbocation resulting from dehydration of the hydroxyl group at C-5 and successive protonation. Since the six-membered ring was

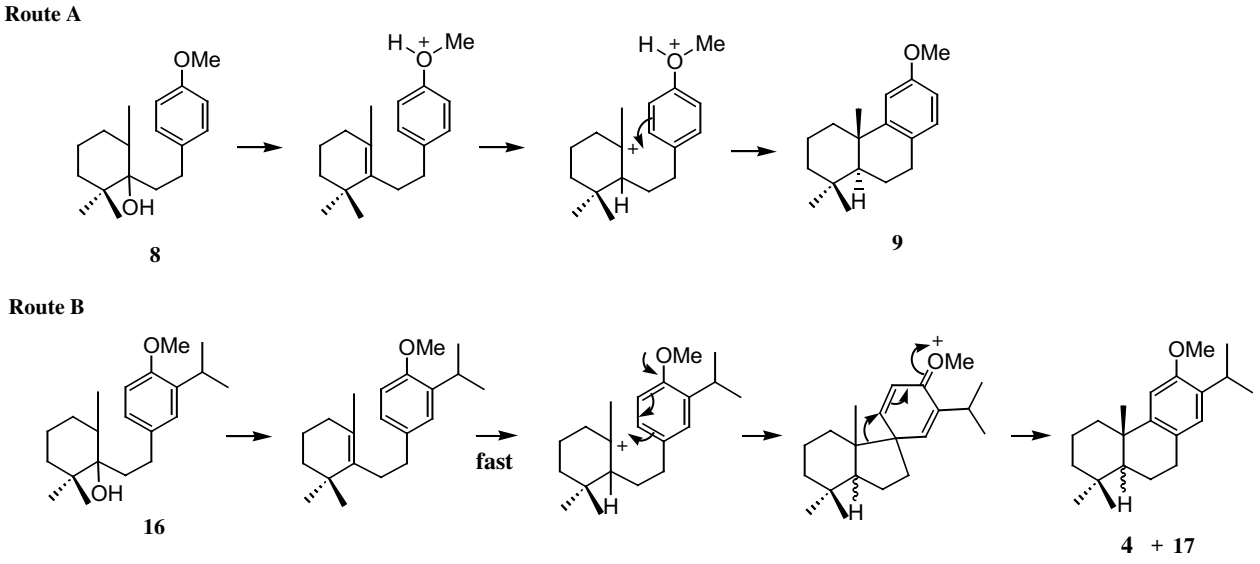


Scheme 2. Synthesis of *O*-methyl ferruginyl ether (**4**). Reagents: (a) CBr_4, Ph_3P; (b) *n*-BuLi; (c) 2,2,6-trimethylcyclohexanone; (d) Pd–C/H_2; (e) Eaton's reagent; (f) AcCl, $AlCl_3$; (g) MeMgBr; (h) $SnCl_4$, Cl_2CHOMe.

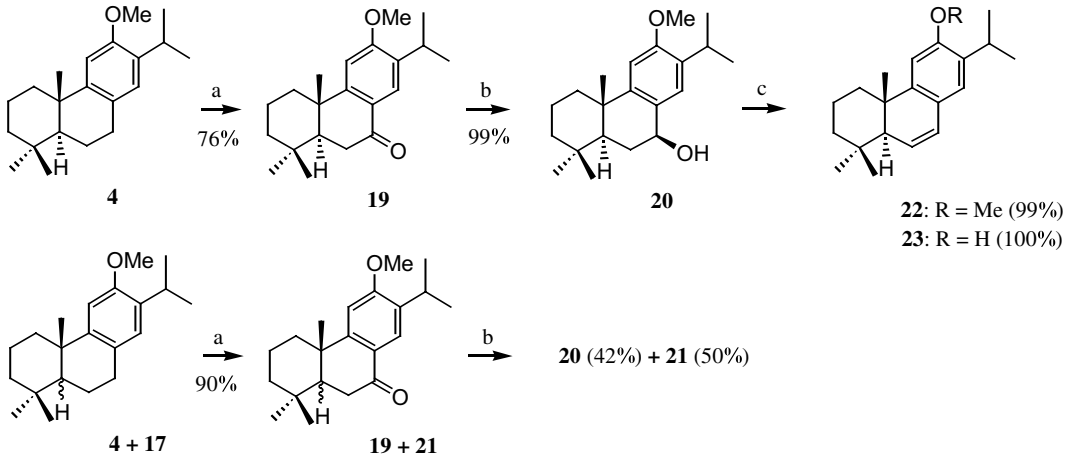
formed as an intermediate in the reaction of **8** with Eaton's reagent, *trans*-fused decaline system was produced prior to *cis*-fused decaline. Meanwhile, a five-membered ring was formed fast in the reaction of **16**, thus, spontaneous dienone–phenol rearrangement afforded both *cis*- and *trans*-isomers (Scheme 3).

Next, ferruginyl methyl ether (**4**) was transformed to the dialdehyde (**2**) according to Cheng's procedure.[36] Oxidation of **4** with chromium trioxide in acetic acid afforded *O*-methyl 7-oxo-ferruginyl ether (**19**), which was reduced with sodium borohydride to give alcohol (**20**). Interestingly, oxidation of a mixture of **4** and **17** with chromium trioxide afforded **19** and its epimer (**21**), which remained intact under the reduction condition with sodium borohydride. The resistance of **21** to the reduction was explained by the steric repulsion between the methyl group at C-4 and newly generated hydroxyl group. Dehydration of **20** with *p*-toluenesulfonic acid was attained in refluxed benzene to give **22**, of which methyl ether was deprotected with odorless dodecanethiol and *n*-butyllithium to afford 6,7-dehydroferruginol (**23**) (Scheme 4).[37,38]

Since ozonolysis of the double bond at C6–C7 in **23** failed to afford **2**, 6,7-dehydroferruginyl methyl ether (**22**) was employed for the ozonolysis. In spite of satisfactory yield in the ozonolysis to afford dialdehyde **24**, deprotection of the methyl ether with lithium *n*-dodecylthiolate did not succeed in obtaining **2** at all. When acetate (**25**) was exposed to the ozonolysis and successive deprotection with potassium carbonate, both dialdehyde

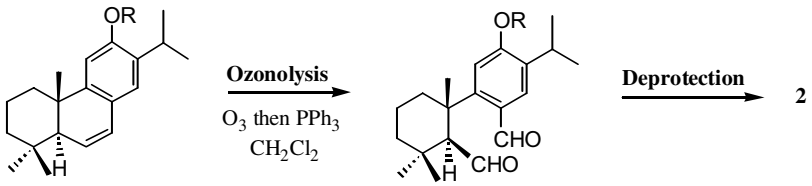


Scheme 3. Mechanism of acid-catalyzed cyclization of the B-ring.



Scheme 4. Transformation of compounds **4** and **17** to compounds **20**–**23**. Reagents: (a) CrO_3; (b) AcOH; (c) *p*-TsOH; (d) Dod-SH, *n*-BuLi.

Table 1. Ozonolysis of abieta-6,8,11,13-tetraene derivatives and successive deprotection



Entry	Starting material	R	Ozonolysis		Deprotection	
			Product	Yield (%)	Condition	Yield (%)
1	**23**	H	**2**	Trace	—	
2	**22**	Me	**24**	70	Dod-SLi/HMPA, 100 °C, 1 h	0
3	**25**	Ac	**26**	85	K_2CO_3/MeOH, rt, 5 h	77
4	**27**	TBS	**28**	89	TBAF/THF, 0 °C, 30 min	91

intermediate (**26**) and **2** were obtained in good yield (Table 1). Finally, the reaction of the ferruginol derivative (**27**) protected with *tert*-butyldimethylsilyl group gave the highest overall yield to afford **2** via **28** (Table 1, entry 4). The obtained dialdehyde (**2**) was not converted to **1** with any Lewis acid such as $AlCl_3$, and $SnCl_4$ while faintly attainable with $BF_3{\cdot}Et_2O$ and well proceeded by protic acid-catalyzed aldol reaction, especially with D-camphorsulfonic acid in CH_2Cl_2, in excellent yield (84%) (Table 2). All of the spectral data of **1** coincided with those in the literature.

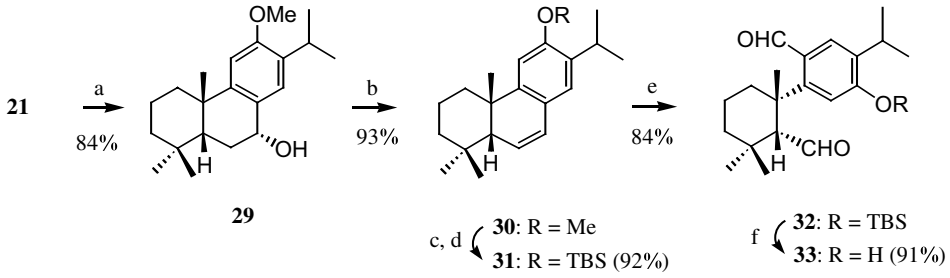
In order to compare the inhibitory activities of the synthetic intermediates of **1** with the corresponding intermediates with A/B *cis*-configuration, the *cis*-isomer (**21**) was reduced with lithium aluminum hydride to afford **29**, which was derived to a dehydrated compound **30** by treatment with *p*-toluenesulfonic acid in benzene under a refluxed condition. After cleavage of the methyl ether in **30** with dodecanethiol in the presence of butyllithium, a newly generated hydroxyl group was reprotected with *tert*-butyldimethylsilyl group to afford **31**. The double bond of **31** was oxidatively cleaved by ozonolysis to give dialdehyde (**32**), of which the protecting group (TBS) was removed with TBAF to yield **33** (Scheme 5).

Finally, evaluation of the some obtained compounds (**1**, **2**, **4**, **17**, **19**, **21**, **30**, and **33**) for aromatase inhibitory

Table 2. Acid-catalyzed aldol reaction from **2** to DL-**1**

2 ⟶ ***dl*-1**

Entry	Reagent/Solvent	Temperature	Time (h)	Yield (%)
1	BF_3Et_2O (1.2 equiv)/CH_2Cl_2	−78 to 0°C	4	8
2	$AlCl_3$ (3.2 equiv)/CH_2Cl_2	−10 °C	4	—
3	$SnCl_4$ (1.2 equiv)/CH_2Cl_2	−78 °C to rt	23	—
4	MsOH (1.2 equiv)/CH_2Cl_2	−10 °C to rt	30	—
5	*p*-TsOH (1.2 equiv)/CH_2Cl_2	rt	1	36
6	(+)-CSA (1.2 equiv)/CH_2Cl_2	rt	8.5	84
7	(+)-CSA (1.2 equiv)/Et_2O	rt	66	8



Scheme 5. Chemical conversion from compound **21** to dial **33**. Reagents: (a) $LiAlH_4$; (b) *p*-TsOH, benzene$NaBH_4$; (c) *n*-BuLi, Dod-SH; (d) TBSCl, imidazole; (e) O_3 then PPh_3; (f) TBAF.

Table 3. Aromatase inhibitory activities of synthesized compounds

Compound	Inhibition[a] (%)			
	300 nM	1 μM	3 μM	10 μM
Ketoconazole	81.7 ± 2.2**	84.2 ± 3.5**	87.5 ± 3.2**	87.9 ± 5.1 **
Letrozole	89.3 ± 1.9**	91.1 ± 6.4**	83.8 ± 3.9**	92.5 ± 6.0 **
Compound **1**	1.1 ± 5.0	10.3 ± 10.0	6.7 ± 6.5	23.7 ± 10.6*
Compound **2**	5.9 ± 7.4	<0	3.3 ± 8.0	9.2 ±11.5
Compound **19**	<0	18.1 ± 10.9*	6.7 ± 5.1	19.8 ± 15.4
Compound **4**	9.0 ± 9.9	2.2 ± 3.8	4.1 ± 9.4	4.8 ± 11.5
Compound **33**	22.2 ± 6.2 **	24.5 ± 7.5 **	24.9 ± 4.3**	25.1 ± 2.6 **
Compound **30**	16.2 ± 2.8**	19.7 ± 5.8**	18.6 ± 10.0*	19.8 ± 6.9**
Compound **21**	20.7 ± 10.0*	28.7 ± 17.5*	23.9 ± 7.9**	19.5 ± 8.6*
Compound **17**	15.2 ± 8.0*	15.9 ± 5.9**	18.4 ± 7.7**	19.7 ± 8.7*

[a] Aromatase inhibitory activity was evaluated by measuring a highly fluorescent product released from 3 μM methoxy-4-trifluoromethyl-coumarin (MFC) at 37 °C for 30 min, in the presence of inhibitors. The blank was realized without adding the human recombinant aromatase enzyme. The results are expressed as % of inhibition to a standard control which was incubated with the human recombinant aromatase enzyme and without inhibitor. Values represent means ± SD of $n = 4$ experiments. Asterisks indicate significant differences from a standard control using Student *t* test.
* $p < 0.05$.
** $p < 0.01$.

activity was performed using a fluorometric substrate and human recombinant aromatase expressed in insect cells with baculovirus system. As shown in Table 3, two typical inhibitors, ketoconazole and letrozole, inhibited aromatase activity significantly ($p < 0.01$), showing this assay for evaluating aromatase inhibitors to be sensitive and reliable. The maximum inhibition with ketoconazole (10 μM) or letrozole (10 μM) was 87.9% or 92.5%, respectively, relative to solvent control. Under the same conditions, no significant ($p < 0.05$) inhibition was found with the compounds **2** and **4** (300 nM–10 μM). In addition, only weak inhibitory activities were observed with compounds **1** and **19**, of which the configurations on the A-ring are *trans* as same as **2** and **4**. Being a good contrast with these results, compounds **17**, **21**, **30**, and **33** having *cis*-configuration on the A ring showed the significant inhibitory effects (Fig. 2).

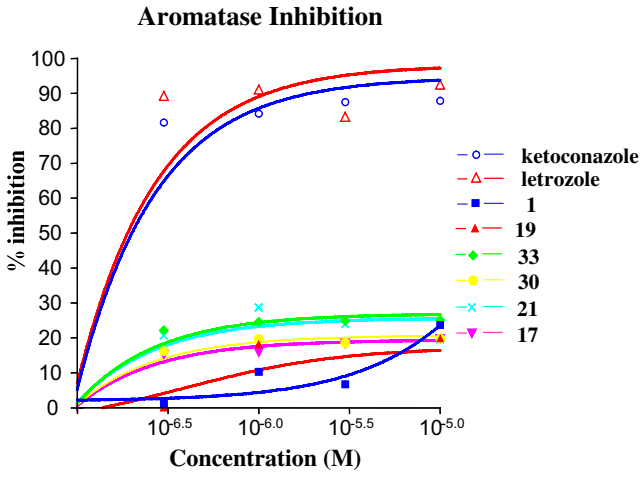


Figure 2. Aromatase inhibition.

3. Conclusion

In conclusion, we have succeeded in the first total synthesis of DL-standishinal (**1**) and found that the structurally related compounds of **1** having the *cis*-configuration on the A-ring showed more potent inhibitory activities than those with the *trans*-configuration against aromatase, of which the excess activity could cause breast cancer.

4. Experimental

4.1. General

Infrared (IR) spectra were recorded on a Shimadzu FTIR-8300 diffraction grating infrared spectrophotometer and ^1H NMR spectra were obtained on a Varian

Unity INOVA 400 spectrometer with tetramethylsilane as an internal standard. ^{13}C NMR spectra were obtained on a Varian Unity INOVA 400 spectrometer with $CDCl_3$ as an internal standard. Mass spectra (MS) were determined on a JEOL JMS-SX 102 A QQ or a JEOL JMS-GC-mate mass spectrometer. Specific rotations were recorded on a Horiba SEPA-200 automatic digital polarimeter. Wakogel C-200 (silica gel) (100–200 mesh, Wako) was used for open column chromatography. Flash column chromatography was performed by using Silica Gel 60N (Kanto Chemical Co., Inc.) as a solid support of immobile phase. Kieselgel 60 F-254 plates (Merck) were used for thin layer chromatography (TLC). Preparative TLC (PTLC) was conducted with Kieselgel 60 F-254 plate (0.25 mm, Merck) or Silica gel 60 F-254 plate (0.5 mm, Merk). Unless purification with silica gel gave compound being pure enough, the compounds were further treated with a recycle HPLC (JAI LC-908) on GPC column (JAIGEL 1H and 2H). In the case it is possible, diastereomeric mixtures were also separated by a recycle HPLC (JAI LC-908) on silica gel column (Kusano Si-10) after the purification mentioned above.

4.2. β-Dibromo-4-methoxystyrene (6)

Carbon tetrabromide (54.5 g, 0.16 mmol) was added to a solution of triphenylphosphine (86.2 g, 0.33 mol) in CH_2Cl_2 at 0 °C. After stirring the mixture for 15 min, 4-methoxybenzaldehyde (**5**) (10 mL, 82.2 mmol) was added dropwise, and then the reaction mixture was stirred for another 30 min at room temperature. After the reaction, the reaction mixture was poured into ice water and extracted with chloroform. The organic layer was successively washed with 5% aqueous solution of sodium thiosulfate and saturated aqueous solution of sodium chloride, dried over $MgSO_4$, and condensed in vacuo. The residue was purified by silica gel column chromatography (hexane/ethyl acetate = 10:0, 30:1, 15:1, and then 10:1) to afford **6** (24.0 g, 99%) as pale yellow crystals; mp: 35–36 °C (*n*-hexane); ^1H NMR (400 MHz, $CDCl_3$) δ: 3.81 (3H, s), 6.87–6.91 (2H, m), 7.40 (1H, s), 7.48–7.52 (2H, m); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 55.3, 87.2, 113.8 (2C), 127.8, 129.9 (2C), 136.3, 159.6; IR ($CHCl_3$): 3029, 3009, 2955, 2839, 1607, 1510, 1248, 1034 cm^{-1}; MS (70 eV) *m*/*z*: 294 (M^++4), 292 (M^++2), 290 (M^+), 279, 277, 275, 132; HRMS calcd for $C_9H_8Br_2$ (M+): 289.8941, found: 289.8939; Anal. Calcd for $C_9H_8Br_2$: C, 37.02; H, 2.76. Found: C, 36.90, H, 2.89.

4.3. 2,2,6-Trimethyl-1-(4-methoxyphenylethynyl)cyclohexanol (7)

n-Butyllithium (2.71 M in hexane, 25.4 ml, 68.7 mmol) was added dropwise into a solution of **6** (10.0 g, 34.4 mmol) in THF (140 ml) at −78 °C, and the mixture was stirred for 1 h and stirred for another 1 h at room temperature. After cooling the reaction mixture at −78 °C again, a solution of 2,2,6-trimethylcyclohexanone (5.06 g, 36.1 mmol) in THF (30 ml) was added to the reaction mixture, the mixture was stirred for 1 h. After the reaction, the mixture was poured into saturated aqueous solution of ammonium chloride, extracted with ethyl acetate. The organic layer was successively washed with 5% aqueous solution of sodium thiosulfate and saturated aqueous solution of sodium chloride, dried over $MgSO_4$, and condensed in vacuo. The residue was purified by silica gel column chromatography (hexane/ethyl acetate = 15:1) to afford **7** (6.33 g, 68%) as colorless crystals; mp: 68–69 °C (petroleum ether); ^1H NMR (400 MHz, $CDCl_3$) δ: 1.05 (3H, s), 1.10 (3H, d, J = 6.4 Hz), 1.18 (3H, s), 1.32–1.48 (3H, m), 1.49–1.55 (1H, m), 1.57–1.63 (1H, m), 1.65–1.72 (1H, m), 1.94 (1H, s), 1.89–1.97 (1H, m), 3.81 (3H, s), 6.82–6.86 (2H, m), 7.36–7.39 (2H, m); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 16.6, 19.9, 21.3, 27.0, 32.9, 37.1, 38.2, 39.2, 55.3, 79.0, 87.0, 88.3, 113.9 (2C), 115.3, 133.0 (2C), 159.5; IR (KBr): 3535, 2978, 2953, 2930, 2359, 1605, 1510, 1452, 1292, 1246 cm^{-1}; MS (70 eV) *m*/*z*: 272 (M+), 239, 201, 159, 121; HRMS calcd for $C_{18}H_{24}O_2$ (M^+): 272.1776, found: 272.1774; Anal. Calcd for $C_{18}H_{24}O_2$: C, 79.37; H, 8.88. Found: C, 79.26; H, 8.70.

4.4. 2,2,6-Trimethyl-1-[2′-(4-methoxyphenyl)ethyl]cyclohexanol (8)

10% Pd–C (ca. 1.0 g) was added to a solution of **7** (6.08 g, 22.3 mmol) in ethanol (80 ml), and the mixture was stirred for 6 h at room temperature under hydrogen atmosphere. After the reaction, the mixture was filtered through Hyflo Super-Cel which was washed with ethyl acetate, and the filtrate was condensed in vacuo. The residue was purified by silica gel column chromatography (hexane/ethyl acetate = 8:1) to afford **8** (6.12 g, 99%) as a colorless oil; ^1H NMR (400 MHz, $CDCl_3$) δ: 0.99 (3H, s), 1.009 (3H, d, J = 7.0 Hz), 1.012 (3H, s), 1.20 (1H, s), 1.14–1.23 (1H, m), 1.31–1.39 (2H, m), 1.41–1.50 (2H, m), 1.56–1.64 (1H, m), 1.73–1.85 (2H, m), 1.92 (1H, ddq, J = 9.5, 7.0, 4.2 Hz), 2.58–2.71 (2H, m), 3.79 (3H, s), 6.82–6.86 (2H, m), 7.12–7.16 (2H, m); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 16.1, 20.3, 23.1, 25.2, 30.4, 30.8, 33.2, 37.68 (2C), 37.74, 39.4, 55.3, 113.8 (2C), 129.2 (2C), 135.5, 157.7; IR ($CHCl_3$): 3007, 2959, 2936, 1611, 1512, 1464, 1240, 1177 cm^{-1}; MS (70 eV) *m*/*z*: 276 (M^+), 258, 134, 121; HRMS calcd for $C_{18}H_{28}O_2$ (M^+): 276.2089, found: 276.2090.

4.5. 12-Methoxypodocarpa-8,11,13-triene (9)

Eaton's reagent (12 g) was added to **8** (5.98 g, 21.6 mmol) and the mixture was stirred for 4 h at room temperature. After the reaction, the reaction mixture was diluted with distilled water, neutralized with aqueous solution of 1 M NaOH, and extracted with ethyl acetate. The organic layer was successively washed with saturated aqueous solution of sodium bicarbonate and saturated aqueous solution of sodium chloride, dried over $MgSO_4$, and condensed in vacuo. The residue was purified by silica gel column chromatography (hexane/ethyl acetate = 40:1) to afford **9** (5.01 g, 90 %) as a colorless oil; ^1H NMR (400 MHz, $CDCl_3$) δ: 0.93, 0.95, 1.19 (each 3H, s), 1.21–1.50 (4H, m), 1.57–1.80 (3H, m), 1.83–1.89 (1H, m), 2.23–2.26 (1H, m), 2.74–2.91 (2H, m), 3.77 (3H, s), 6.66 (1H, dd, J = 8.4, 2.7 Hz), 6.81 (1H, d, J = 2.7 Hz), 6.96 (1H, d, J = 8.4 Hz); ^{13}C NMR (100 MHz, $CDCl_3$)

δ: 19.1, 19.3, 21.6, 24.7, 19.5, 33.3, 33.5, 38.0, 38.8, 41.6, 50.3, 55.2, 110.1, 110.7, 127.5, 129.7, 151.4, 157.6; IR ($CHCl_3$): 2930, 2361, 1609, 1574, 1502, 1491, 1252 cm^{-1}; MS (70 eV) *m/z*: 258 (M^+), 243, 227, 187, 173, 161, 147; HRMS calcd for $C_{18}H_{26}O$ (M^+): 258.1948, found: 258.1985.

4.6. 13-Acetyl-12-methoxypodocarpa-8,11,13-triene (10)

Aluminum chloride (5.59 g, 41.9 mmol) and acetyl chloride (3.18 ml, 44.7 mmol) were added to a solution of **9** (4.81 g, 18.6 mmol) in CH_2Cl_2 at −10 °C, and the reaction mixture was stirred for 2 h at −5 °C. After the reaction, the reaction mixture was poured into ice water and extracted with ethyl acetate. The organic layer was successively washed with saturated aqueous solution of sodium bicarbonate and saturated aqueous solution of sodium chloride, dried over $MgSO_4$, and condensed in vacuo. The residue was purified by silica gel column chromatography (hexane/ethyl acetate = 10:1) to afford **10** (5.73 g, 97%) as a colorless oil; ^1H NMR (400 MHz, $CDCl_3$) δ: 0.93, 0.95, 1.20 (each 3H, s), 1.15–1.32 (2H, m), 1.39–1.52 (2H, m), 1.61–1.80 (3H, m), 1.85–1.91 (1H, m), 2.24–2.28 (1H, m), 2.58 (3H, s), 2.67–2.94 (2H, m), 3.87 (3H, s), 6.83 (1H, s), 7.45 (1H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 18.9, 19.2, 21.7, 24.5, 29.2, 31.8, 33.2, 33.5, 38.5, 38.8, 41.5, 50.0, 55.4, 107.5, 125.5, 127.5, 130.9, 156.4, 157.2, 199.5; IR ($CHCl_3$): 3007, 2930, 2868, 1668, 1605, 1495, 1464, 1404, 1267, 1240 cm^{-1}; MS (70 eV) *m/z*: 300 (M^+), 257, 243, 149; HRMS calcd for $C_{20}H_{28}O_2$ (M^+): 300.2089, found: 300.2094.

4.7. 12-Methoxyabieta-8,11,13-trien-15-ol (11)

Methyl magnesium bromide (3.0 M in diethyl ether, 12.2 ml, 36.5 mmol) was added to a solution of **10** (5.48 g, 18.2 mmol) in THF (200 ml) at 0 °C and the reaction mixture was stirred for 4 h. After the reaction, the reaction mixture was poured into saturated aqueous solution of ammonium chloride and extracted with ethyl acetate. The organic layer was washed with saturated aqueous solution of sodium chloride, dried over $MgSO_4$, and condensed in vacuo. The residue was purified by silica gel column chromatography (hexane/ethyl acetate = 7:1) to afford **11** (5.66 g, 98%) as a colorless oil; ^1H NMR (400 MHz, $CDCl_3$) δ: 0.93, 0.95, 1.19 (each 3H, s), 1.18–1.25 (1H, m), 1.30–1.34 (1H, m), 1.38–1.54 (2H, m), 1.57 (3H, s), 1.58 (3H, s), 1.61–1.65 (1H, m), 1.66–1.82 (2H, m), 1.84–1.90 (1H, m), 2.22–2.25 (1H, m), 2.72–2.89 (2H, m), 3.87 (3H, s), 4.22 (1H, s), 6.78 (1H, s), 6.92 (1H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 19.1, 19.3, 21.6, 24.7, 29.7, 29.8, 29.9, 33.3, 33.4, 37.8, 38.8, 41.6, 50.3, 55.3, 72.2, 107.3, 126.2, 127.2, 132.9, 149.7, 155.0; IR ($CHCl_3$): 3533, 3007, 2968, 2930, 2359, 1612, 1501, 1464, 1385, 1369, 1244 cm^{-1}; MS (70 eV) *m/z*: 316 (M^+), 302, 301, 285, 243, 201, 161; HRMS calcd for $C_{21}H_{32}O_2$ (M^+): 316.2402, found: 316.2403.

4.8. *O*-Methyl ferruginyl ether (4)

10% Pd–C (ca. 578 mg) was added to a solution of **11** (5.39 g, 17.0 mmol) in methanol (50 ml) and acetic acid (50 ml), and the mixture was stirred at 50 °C for 8 h under hydrogen atmosphere. After the reaction, the mixture was filtered through Hyflo Super-Cel which was washed with ethyl acetate, and the filtrate was condensed in vacuo. An aqueous solution of the residue was neutralized with an aqueous solution of sodium bicarbonate and extracted with ethyl acetate. The organic layer was washed with saturated aqueous solution of sodium chloride, dried over $MgSO_4$, and condensed in vacuo. The residue was purified by silica gel column chromatography (hexane/ethyl acetate = 100:1) to afford **4** (4.73 g, 92%) as a colorless oil; ^1H NMR (400 MHz, $CDCl_3$) δ: 0.92, 0.94, 1.17 (each 3H, s), 1.19 (6H, d, *J* = 7.0 Hz), 1.15–1.25 (1H, m), 1.32–1.51 (3H, m), 1.58–1.78 (3H, m), 1.83–1.89 (1H, m), 2.23–2.27 (1H, m), 2.73–2.89 (2H, m), 3.21 (1H, septet, *J* = 7.0 Hz), 3.79 (3H, s), 6.72, 6.48 (each 1H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 19.2, 19.3, 21.6, 22.7, 22.9, 24.8, 26.4, 29.8, 33.3, 33.4, 37.8, 38.9, 41.7, 50.5, 55.6, 106.5, 126.4, 126.9, 134.1, 148.0, 155.0; IR ($CHCl_3$): 3001, 2963, 2930, 2868, 1711, 1611, 1499, 1463, 1364, 1250 cm^{-1}; MS (70 eV) *m/z*: 300 (M^+), 285, 257, 243, 229, 215, 203, 189, 163; HRMS calcd for $C_{21}H_{32}O$ (M^+): 300.2453, found: 300.2457.

4.9. 3-Isopropyl-4-methoxybenzaldehyde (12)

Tin (IV) chloride (27.9 g, 0.11 mol) and dichloromethoxymethane (16.4 g, 0.14 mol) were added to a solution of 2-isopropylanisole (**13**) (10.7 g, 71.3 mmol) in CH_2Cl_2 (160 ml) at 0 °C, and the mixture was stirred for 1 h. After the reaction, the reaction mixture was poured into ice water and extracted with chloroform. The organic layer was washed with saturated aqueous solution of sodium chloride, dried over $MgSO_4$, and condensed in vacuo. The residue was purified by silica gel column chromatography (hexane/ethyl acetate = 6:1) to afford **12** (12.1 g, 95%) as a pale yellow oil; ^1H NMR (400 MHz, $CDCl_3$) δ: 1.24 (6H, d,*J* = 7.0 Hz), 3.33 (1H, septet, *J* = 7.0 Hz), 3.92 (3H, s), 6.95 (1H, d, *J* = 8.4 Hz), 7.71 (1H, dd, *J* = 8.4, 2.0 Hz), 7.77 (1H, d, *J* = 2.0 Hz), 10.46 (1H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 22.4, 26.7, 55.6, 110.0, 127.2, 129.7, 130.5, 137.9, 162.0, 191.3; IR ($CHCl_3$): 3030, 3018, 3007, 2966, 2841, 2741, 1686, 1599, 1580, 1502, 1493, 1462, 1261, 1171 cm^{-1}; MS (70 eV) *m/z*: 178 (M^+), 163, 149; HRMS calcd for $C_{11}H_{14}O_2$ (M^+): 178.0094, found: 178.0092.

4.10. β-Dibromo-3-isopropyl-4-methoxystyrene (14)

A solution of **12** (12.0 g, 67.4 mmol) in CH_2Cl_2 (50 ml) was added to a mixture prepared by adding carbon tetrabromide (44.7 g, 0.13 mol) into a solution of triphenylphosphine (70.7 g, 0.27 mol) in CH_2Cl_2 (200 ml) at 0 °C. After stirring the reaction mixture for 2.5 h at room temperature, the reaction mixture was poured into ice water and extracted with chloroform. The organic layer was successively washed with 5% aqueous solution of sodium thiosulfate and saturated aqueous solution of sodium chloride, dried over $MgSO_4$, and condensed in vacuo. The residue was purified by silica gel column chromatography (hexane) to afford **14** (23.1 g, 99%) as a colorless oil; ^1H NMR (400 MHz, $CDCl_3$) δ: 1.20

(6H, d, J = 6.9 Hz), 3.31 (1H, septet, J = 6.9 Hz), 3.84 (3H, s), 6.82–6.84 (1H, m), 7.39–7.42 (3H, m); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 22.6 (2C), 26.2, 55.4, 86.7, 110.0, 126.6, 127.0, 127.5, 136.8, 136.9, 156.9; IR ($CHCl_3$): 3032, 3007, 2964, 2870, 1603, 1497, 1464, 1250, 1032 cm^{-1}; MS (70 eV) m/z: 334 (M^++2), 319, 304, 238, 174, 159, 144; HRMS calcd for $C_{12}H_{14}Br_2O$ (M^+): 331.9411, found: 331.9415.

4.11. 2,2,6-Trimethyl-1-(3-isopropyl-4-methoxyphenylethynyl)cyclohexanol (15)

n-Butyllithium (2.64 M in hexane, 7.12 ml, 18.8 mmol) was added to a solution of **14** (3.14 g, 9.40 mmol) in THF (40 ml) at −78 °C, and the mixture was stirred for 1 h with keeping the temperature and for another 1 h at room temperature. After cooling the mixture at −78 °C again, a solution of 2,2,6-trimethylcyclohexanone (1.45 g, 10.3 mmol) in THF (10 ml) was added to the reaction mixture. Stirring at −78 °C for 2 h, the reaction mixture was poured into saturated aqueous solution of ammonium chloride and extracted with ethyl acetate. The organic layer was successively washed with 5% aqueous solution of sodium thiosulfate and saturated aqueous solution of sodium chloride, dried over $MgSO_4$, and condensed in vacuo. The residue was purified by silica gel column chromatography (hexane/ethyl acetate = 20:1) to afford diastereomixture of **15** (2.37 g, 80%) as a colorless oil; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.05 (3H, s), 1.11 (3H, d, J = 6.4 Hz), 1.19 (3H, s), 1.20 (6H, d, J = 6.9 Hz), 1.18–1.21 (1H, m), 1.33–1.38 (1H, m), 1.40–1.55 (2H, m), 1.57–1.64 (1H, m), 1.67–1.74 (1H, m), 1.94 (1H, s), 1.89–1.99 (1H, m), 3.28 (1H, septet, J = 6.9 Hz), 3.83 (3H, s), 6.76–6.78 (1H, m), 7.25–7.28 (2H, m); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 16.7, 19.9, 21.3, 22.5 (2C), 26.7, 27.0, 32.9, 37.1, 38.2, 39.2, 55.4, 79.1, 87.6, 87.7, 110.1, 115.0, 129.4, 130.3, 137.1, 156.9; IR ($CHCl_3$): 3609, 3007, 2964, 2220, 1603, 1495, 1464, 1250, 1175, 1032 cm^{-1}; MS (70 eV) m/z: 314 (M^+), 299, 296, 281, 257, 243, 229, 215, 163; HRMS cal;cd for $C_{21}H_{30}O_2$ (M^+): 314.2246, found: 314.2247.

4.12. 2,2,6-Trimethyl-1-[2′-(3-isopropyl-4-methoxyphenyl)ethyl]cyclohexanol (16)

10% Pd–C (ca. 20 mg) was added to a solution of **15** (84.2 mg, 0.27 mmol) in methanol (3 ml) and the mixture was stirred for 2 h at room temperature under hydrogen atmosphere. After the reaction, the reaction mixture was filtered through Hyflo Super-Cel that was successively washed with ethyl acetate. The filtrate was condensed in vacuo and the residue was purified by silica gel column chromatography (hexane/ethyl acetate = 10:1) to afford **16** (85 mg, 99%) as a colorless oil; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 0.99, 1.017 (each 3H, s), 1.018 (3H, d, J = 7.0 Hz), 1.22 (6H, d, J = 6.9 Hz), 1.23 (1H, s), 1.19–1.23 (1H, m), 1.34–1.39 (2H, m), 1.44–1.50 (2H, m), 1.57–1.63 (1H, m), 1.76–1.85 (2H, m), 1.89–1.96 (1H, m), 2.58–2.70 (2H, m), 3.30 (1H, septet, J = 6.9 Hz), 3.81 (3H, s), 6.78 (1H, d, J = 8.2 Hz), 7.01 (1H, dd, J = 8.2, 2.3 Hz), 7.04 (1H, d, J = 2.3 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 16.1, 20.3, 22.7 (2C), 23.2, 25.2, 26.8, 30.6, 30.8, 33.2, 37.7 (2C), 39.4, 55.5, 76.8, 110.4, 126.0, 126.2, 135.2, 136.9, 155.0; IR ($CHCl_3$): 3612, 3007, 2963, 2870, 1497, 1464, 1244, 1171, 1034 cm^{-1}; MS (70 eV) m/z: 318 (M^+), 300, 176, 163; HRMS calcd for $C_{21}H_{34}O_2$ (M^+): 318.2559, found: 318.2560.

4.13. 12-Methoxy-5β-abieta-8,11,13-triene (17) and ferruginyl methyl ether (4)

Eaton's reagent (3 g) was added to **16** (1.58 g, 4.96 mmol) and the mixture was stirred for 15 min at room temperature. After the reaction, the reaction mixture was poured into ice water. The aqueous solution was neutralized with an aqueous solution of 1 M NaOH and extracted with ethyl acetate. The organic layer was successively washed with a saturated aqueous solution of sodium bicarbonate and a saturated aqueous solution of sodium chloride, dried over $MgSO_4$, and condensed in vacuo. The residue was purified by silica gel column chromatography (hexane) to afford **4** (545 mg, 37%) and **17** (870 mg, 58%) as a colorless oil; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 0.39, 0.93, 1.171 (each 3H, s), 1.177 (3H, d, J = 6.9 Hz), 1.184 (3H, d, J= 6.9 Hz), 1.24–1.53 (6H, m), 1.90–1.97 (1H, m), 2.11–2.21 (1H, m), 2.33–2.37 (1H, m), 2.72–2.85 (2H, m), 3.22 (1H, septet, J = 6.9 Hz), 3.80 (3H, s), 6.74 (1H, s), 6.79 (1H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 18.4, 19.4, 22.86, 22.89, 23.0, 25.7, 26.4, 32.7, 34.2, 34.5, 37.3, 38.2, 43.0, 50.3, 55.6, 106.4, 126.2, 128.9, 133.8, 141.6, 154.8; IR ($CHCl_3$): 2961, 2937, 2864, 1612, 1502, 1464, 1244 cm^{-1}; MS (70 eV) m/z: 300 (M^+), 285, 257, 243, 215, 203, 189, 163; HRMS calcd for $C_{21}H_{32}O$ (M^+): 300.2453, found: 300.2450.

4.14. *O*-Methyl sugiyl ether (19)

A solution of chromium trioxide (168 mg, 1.68 mmol) in acetic acid (5 ml) was added to a solution of **4** (420 mg, 1.40 mmol) in acetic acid (15 ml), and the mixture was stirred for 2 h at room temperature. After the reaction, the reaction mixture was poured into ice water. The aqueous solution was neutralized with an aqueous solution of 1 M NaOH and extracted with ethyl acetate. The organic layer was successively washed with saturated aqueous solution of sodium bicarbonate and saturated aqueous solution of sodium chloride, dried over $MgSO_4$, and condensed in vacuo. The residue was purified by silica gel column chromatography (hexane/benzene = 1:2) to afford **19** (332 mg, 76%) as colorless crystals; mp: 132–133 °C (MeOH); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 0.93, 1.00 (each 3H, s), 1.19 (3H, d, J = 6.9 Hz), 1.22 (3H, d, J = 6.9 Hz), 1.25 (3H, d, J = 0.5 Hz), 1.23–1.31 (1H, m), 1.51–1.61 (2H, m), 1.66–1.72 (1H, m), 1.74–1.83 (1H, m), 1.88 (1H, dd, J = 13.5, 4.2 Hz), 2.28–2.32 (1H, m), 2.60 (1H, dd, A part of AB, J = 18.1, 13.5 Hz), 2.68 (1H, dd, B part of AB, J = 18.1, 4.2 Hz), 3.24 (1H, septet, J = 6.9 Hz), 3.89 (3H, s), 6.75 (1H, s), 7.89 (1H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 18.9, 21.4, 22.4, 22.5, 23.2, 26.5, 32.6, 33.3, 36.0, 38.0, 38.3, 41.3, 49.6, 55.4, 104.4, 124.1, 125.6, 135.2, 156.4, 161.6, 198.5; IR ($CHCl_3$): 3483, 3005, 2964, 2932, 1663, 1597, 1562, 1495, 1277, 1261 cm^{-1}; MS (70 eV)

m/z: 314 (M^+), 299, 257, 177, 149; HRMS calcd for $C_{21}H_{30}O_2$ (M^+): 314.2246, found: 314.2241; Anal. Calcd for $C_{21}H_{30}O_2$: C, 80.21; H, 9.62. Found: C, 80.11; H, 9.65.

4.15. 12-Methoxyabieta-8,11,13-trien-7β-ol (20)

A solution of sodium borohydride (3 mg, 0.08 mmol) in methanol (0.5 ml) was added to a solution of **19** (21 mg, 0.07 mmol) in methanol (1 ml) and the mixture was stirred for 13 h at room temperature. After the reaction, the reaction mixture was diluted with water and extracted with diethyl ether. The organic layer was washed with saturated aqueous solution of sodium chloride, dried over $MgSO_4$, and condensed in vacuo. The residue was purified by preparative thin layer chromatography (hexane/ethyl acetate = 5:1) to afford **20** (21 mg, 99%) as colorless crystals; mp: 128–130 °C (hexane); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 0.94, 0.95 (each 3H, s), 1.19, 1.21 (each 3H, d, J = 6.8 Hz), 1.14–1.25 (1H, m), 1.28 (3H, s), 1.33–1.42 (2H, m), 1.46–1.51 (1H, m), 1.57–1.68 (2H, m), 1.70–1.81 (2H, m), 2.22–2.28 (2H, m), 3.24 (1H, septet, J = 6.8 Hz), 3.80 (3H, s), 4.48 (1H, br s), 6.68 (1H, s), 7.33 (1H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 19.2, 21.5, 22.68, 22.72, 25.2, 26.6, 30.4, 33.2 (2C), 38.5, 38.9, 41.3, 49.4, 55.4, 71.0, 106.0, 124.9, 129.9, 134.8, 148.4, 156.3; IR ($CHCl_3$): 3591, 3442, 3003, 2961, 2932, 2868, 1609, 1568, 1501, 1464, 1248 cm^{-1}; MS (70 eV) *m/z*: 316 (M^+), 273, 241, 192, 177; HRMS calcd for $C_{21}H_{32}O_2$ (M^+): 316.2402, found, 316.2399; Anal. Calcd for $C_{21}H_{32}O_2$: C, 79.70; H, 10.19. Found: C, 79.69; H, 10.10.

4.16. Oxidation of ferruginyl methyl ether (4) and 17

A solution of chromium trioxide (0.37 g, 3.72 mmol) in acetic acid (10 ml) was added to a solution of **4** and **17** (932 mg, 3.10 mmol) in acetic acid (20 ml), and the mixture was stirred for 4 h at room temperature. After the reaction, the reaction mixture was poured into ice water. The aqueous solution was neutralized with an aqueous solution of 1 M NaOH and extracted with ethyl acetate. The organic layer was successively washed with saturated aqueous solution of sodium bicarbonate and saturated aqueous solution of sodium chloride, dried over $MgSO_4$, and condensed in vacuo. The residue was purified by silica gel column chromatography (hexane/benzene = 1:2) to afford a mixture of **19** and **21** (880 mg, 90 %).

4.17. Reduction of a mixture of 19 and 21

A solution of sodium borohydride (5 mg, 0.14 mmol) was added to a solution of **19** and **21** (35.7 mg, 0.11 mmol) in methanol (1 ml) at 0 °C and the mixture was stirred for 13 h at room temperature. After the reaction, the reaction mixture was diluted with water and extracted with diethyl ether. The organic layer was washed with saturated aqueous solution of sodium chloride, dried over $MgSO_4$, and condensed in vacuo. The residue was purified by preparative thin layer chromatography (hexane/ethyl acetate = 8:1) to afford **20** (15.2 mg, 42%) and **21** (17.9 mg, 50%), which is recovered as an intact substrate.

4.18. 12-Methoxy-5β-abieta-8,11,13-trien-7-one (21)

Colorless crystals; mp: 152–154 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 0.31, 0.94 (each 3H, s), 1.21 (3H, d, J = 6.8 Hz), 1.22 (3H, d, J = 7.0 Hz), 1.29 (3H, s), 1.31–1.39 (2H, m), 1.41–1.52 (3H, m), 1.82–1.84 (1H, m), 2.43–2.47 (1H, m), 2.76 (1H, d, A part of AB, J = 19.0 Hz), 2.96 (1H, dd, B part of AB, J = 19.0, 7.0 Hz), 3.24 (1H, septet, J = 7.0 Hz), 3.91 (3H, s), 6.73 (1H, s), 7.90 (1H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 19.2, 22.4, 22.5, 22.8, 26.6, 31.9, 34.5, 35.5, 36.1, 37.5, 37.9, 42.5, 52.2, 105.1, 125.7, 126.3, 135.0, 149.5, 161.7, 197.6; IR (CHCl3): 3462, 3007, 2964, 2936, 2868, 1657, 1597, 1560, 1499, 1271, 1254 cm^{-1}; MS (70 eV) *m/z*: 314 (M^+), 299, 257, 149; HRMS calcd for $C_{21}H_{30}O_2$: 314.2246, found: 314.2247.

4.19. 6,7-Dehydroferruginyl methyl ether (22)

p-Toluenesulfonic acid (0.23 g, 1.21 mmol) was added to a solution of **20** (3.83 g, 12.1 mmol) in benzene, and the mixture was refluxed for 1 h. After the reaction, the reaction mixture was poured into a saturated aqueous solution of sodium bicarbonate and extracted with ethyl acetate. The organic layer was washed with a saturated aqueous solution of sodium chloride, dried over $MgSO_4$, and condensed in vacuo. The residue was purified by silica gel column chromatography (hexane/ethyl acetate = 50:1) to afford **22** (3.60 g, 99%) as a colorless oil; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 0.97 (3H, s), 1.03 (3H, d, J = 0.5 Hz), 1.05 (3H, s), 1.17 (3H, d, J = 6.9 Hz), 1.21 (3H, d, J = 6.9 Hz), 1.19–1.27 (1H, m), 1.50–1.55 (1H, m), 1.58–1.84 (3H, m), 2.10 (1H, dd, J = 3.1, 2.6 Hz), 2.15–2.18 (1H, m), 3.25 (1H, septet, J = 6.9 Hz), 3.83 (3H, s), 5.89 (1H, dd, J = 9.5, 2.6 Hz), 6.50 (1H, dd, J = 9.5, 3.1 Hz), 6.69 (1H, s), 6.90 (1H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 19.0, 20.1, 22.5 (2C), 22.9, 26.3, 32.6, 32.9, 36.1, 37.9, 41.1, 51.1, 55.6, 104.7, 124.3, 125.8, 127.3, 127.4, 133.8, 147.0, 156.3; IR ($CHCl_3$): 3003, 2961, 2930, 2868, 1603, 1556, 1495, 1464, 1258 cm^{-1}; MS (70 eV) *m/z*: 298 (M^+), 283, 227, 216, 199, 173; HRMS calcd for $C_{21}H_{30}O$ (M^+): 298.2297, found: 298.2296.

4.20. 6,7-Dehydroferruginol (23)

n-Butyllithium (2.71 M in hexane, 41.9 ml, 0.11 mol) was added to a solution of dodecanethiol (27.2 ml, 0.11 mol) in HMPA (50 ml) and the mixture was stirred for 30 min at room temperature. To the mixture **22** (3.39 g, 11.4 mmol) was added and the reaction mixture was stirred for 5 h at 100 °C. After the reaction, the reaction mixture was poured into 1 M hydrochloric acid and extracted with diethyl ether. The organic layer was successively washed with distilled water and saturated aqueous solution of sodium chloride, dried over $MgSO_4$, and condensed in vacuo. The residue was purified by silica gel column chromatography (hexane/ethyl acetate = 15:1) to afford **23** (3.23 g, 100%) as a colorless oil; ^{1}H NMR (100 MHz, $CDCl_3$) δ: 0.96, 1.01, 1.04 (each 3H, s), 1.23 (3H, d, J = 6.9 Hz), 1.26 (3H, d, J = 6.9 Hz), 1.25–1.29 (1H, m), 1.49–1.78 (5H, m), 2.05–2.10 (1H, m), 3.13 (1H, septet, J = 6.9 Hz), 4.69 (1H, s), 5.87 (1H, dd, J = 9.5, 2.7 Hz), 6.49 (1H, dd,

J = 9.5, 3.1 Hz), 6.58, 6.89 (each 1H, s); ^{13}C NMR (100 MHz, $CDCl_3$) d: 19.0, 20.1, 22.4, 22.5, 22.8, 26.7, 32.6, 32.8, 36.0, 37.6, 41.0, 50.9, 109.5, 124.5, 126.3, 127.6, 130.9, 147.5, 152.1; IR ($CHCl_3$): 3599, 3341, 3003, 2963, 2930, 2870, 1730, 1605, 1460, 1265 cm^{-1}; MS (70 eV) *m/z*: 284 (M^+), 213, 202, 185, 159; HRMS calcd for $C_{20}H_8O$ (M^+): 284.2140, found: 284.2137.

4.21. 12-Methoxy-6,7-secoabieta-8,11,13-trien-6,7-dial (24)

Ozone gas was passed through a solution of **22** (102 mg, 0.34 mmol) in CH_2Cl_2 (20 ml) at −78 °C until pale blue color of the solution maintained. After the reaction, excess amount of ozone was released by bubbling nitrogen gas. To the reaction mixture, a solution of triphenylphosphine (0.18 g, 0.68 mmol) in CH_2Cl_2 (5 ml) was added and the mixture was stirred for 1 h at −78 °C and for another 2 h at room temperature. The organic solvent of the reaction mixture was evaporated and the residue was purified by silica gel column chromatography (hexane/ethyl acetate = 8:1) to afford **24** (78.9 mg, 70%) as a pale yellow oil; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 0.67, 1.01 (each 3H, s), 1.16–1.22 (1H, m), 1.22, 1.23 (each 3H, d, J = 7.0 Hz), 1.54 (3H, s), 1.63–1.70 (1H, m), 1.73–1.87 (3H, m), 2.33–2.39 (1H, m), 3.12 (1H, d, J = 4.2 Hz), 3.26 (1H, septet, J = 7.0 Hz), 3.93 (3H, s), 6.99, 7.83 (each 1H, s), 9.89 (1H, d, J = 4.2 Hz), 10.55 (1H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 19.4, 22.2, 22.3, 26.4, 30.4, 33.5, 37.6, 40.8, 55.4 (2C), 65.0, 77.2, 101.2, 109.1, 127.5, 133.0, 134.9, 151.0, 160.7, 191.4, 205.6; IR ($CHCl_3$): 2963, 2936, 2872, 2361, 2340, 1715, 1670, 1597, 1464, 1254 cm^{-1}; MS (70 eV) *m/z*: 330 (M^+), 301, 287, 217, 161; HRMS calcd for $C_{21}H_{30}O_3$ (M^+): 330.2195, found: 330.2194.

4.22. 12-Acetoxyabieta-6,8,11,13-tetraene (25)

Acetic anhydride (0.37 ml, 3.90 mmol) and DMAP (16 mg, 1.30 mmol) were added to a solution of **23** (370 mg, 1.30 mmol) in pyridine (10 ml) and the mixture was stirred for 6 h at room temperature. After the reaction, the reaction mixture was diluted with ice water, the pH value of the aqueous solution was adjusted at 4.0 with 1 M hydrochloric acid, and extracted with ethyl acetate. The organic layer was washed with saturated aqueous solution of sodium chloride, dried over $MgSO_4$, and condensed in vacuo. The residue was purified by silica gel column chromatography (hexane/ethyl acetate = 20:1) to afford **25** (416 mg, 98%) as a colorless oil; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 0.96, 1.038, 1.042 (each 3H, s), 1.17 (3H, d, J = 6.9 Hz), 1.21 (3H, d, J = 6.9 Hz), 1.19–1.26 (1H, m), 1.49–1.77 (4H, m), 2.05–2.08 (1H, m), 2.13 (1H, dd, J = 3.1, 2.7 Hz), 2.31 (3H, s), 2.94 (1H, septet, J = 6.9 Hz), 5.99 (1H, dd, J = 9.5, 2.7 Hz), 6.53 (1H, dd, J = 9.5, 3.1 Hz), 6.76, 6.97 (each 1H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 18.9, 20.2, 22.5, 22.8, 23.1, 27.1, 32.5, 32.8, 35.9, 37.7, 40.9, 50.7, 116.1, 116.2, 124.5, 127.1, 130.1, 131.1, 136.9, 147.1, 169.8; IR ($CHCl_3$): 2963, 2930, 2361, 2341, 1751, 1489, 1458, 1371, 1190 cm^{-1}; MS (70 eV) *m/z*: 326 (M^+), 284, 269, 202, 185, 159; HRMS calcd for $C_{22}H_{30}O_2$ (M^+): 326.2246, found: 326.2244.

4.23. 12-Acetoxy-6,7-secoabieta-8,11,13-trien-6,7-dial (26)

Ozone gas was passed through a solution of **25** (300 mg, 0.92 mmol) in CH_2Cl_2 (30 ml) at −78 °C until pale blue color of the solution maintained. After the reaction, excess amount of ozone was released by bubbling nitrogen gas. To the reaction mixture, a solution of triphenylphosphine (0.48 g, 1.84 mmol) in CH_2Cl_2 (10 ml) was added and the mixture was stirred for 1 h at −78 °C and for another 1 h at room temperature. The organic solvent of the reaction mixture was evaporated and the residue was purified by silica gel column chromatography (hexane/ethyl acetate = 5:1) to afford **26** (283 mg, 81%) as a pale yellow oil; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 0.58 (3H, s), 0.96 (6H, s), 1.23 (3H, d, J = 6.8 Hz), 1.24 (3H, d, J = 6.8 Hz), 1.25–1.29 (1H, m), 1.39–1.52 (2H, m), 1.65–1.85 (2H, m), 2.37 (3H, s), 2.30–2.39 (1H, m), 2.91 (1H, d, J = 4.7 Hz), 3.03 (1H, septet, J = 6.8 Hz), 7.16 (1H, s), 7.92 (1H, s), 9.93 (1H, d, J = 4.7 Hz), 10.73 (1H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 19.1, 21.0, 22.60 (2C), 22.63 (2C), 27.2, 29.8, 33.6, 36.9, 37.2, 40.2, 66.0, 121.6, 132.1, 133.1, 138.6, 149.4, 151.9, 169.0, 191.5, 205.2; IR ($CHCl_3$): 2964, 2936, 2359, 1757, 1715, 1682, 1371, 1190, 1171 cm^{-1}; MS (70 eV) *m/z*: 358 (M^+), 301, 245, 203, 189; HRMS calcd for $C_{22}H_{30}O_4$ (M^+): 358.2144, found: 358.2139.

4.24. 12-(*tert*-Butyldimethylsilyloxy)abieta-6,8,11,13-tetraene (27)

tert-Butyldimethylsilyl chloride (0.30 g, 1.97 mmol) and imidazole (0.27 g, 3.93 mmol) were added to a solution of **23** (280 mg, 0.98 mmol) in DMF (10 ml) and the mixture was stirred for 19 h at room temperature. After the reaction, the reaction mixture was poured into a saturated aqueous solution of ammonium chloride and extracted with diethyl ether. The organic layer was washed with a saturated aqueous solution of sodium chloride, dried over $MgSO_4$, and condensed in vacuo. The residue was purified by silica gel column chromatography (hexane/ethyl acetate = 50:1) to afford **27** (383 mg, 98%) as a colorless oil; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 0.23, 0.24, 0.96 (each 3H, s), 1.02 (12H, s), 1.04 (3H, s), 1.16 (3H, d, J = 6.9 Hz), 1.20 (3H, d, J = 6.9 Hz), 1.19–1.27 (1H, m), 1.50–1.60 (2H, m), 1.65–1.78 (2H, m), 2.04–2.09 (1H, m), 2.09 (1H, dd, J = 3.1, 2.7 Hz), 3.24 (1H, septet, J = 6.9 Hz), 5.87 (1H, dd, J = 9.5, 2.7 Hz), 6.49, 6.88 (1H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ: −4.1, −3.9, 18.3, 19.1, 20.2, 22.5, 22.7, 23.0, 25.9 (3C), 26.2, 32.6, 32.8, 36.0, 37.6, 41.1, 51.0, 112.4, 124.3, 126.2, 127.5, 135.6, 146.7, 152.2; IR ($CHCl_3$): 3001, 2961, 2930, 2862, 1603, 1551, 1493, 1275, 1256, 1175 cm^{-1}; MS (70 eV) *m/z*: 398 (M^+), 383, 341, 316, 273, 257, 153; HRMS calcd for $C_{26}H_{42}OSi$ (M^+): 398.3005, found 398.3004.

4.25. 12-(*tert*-Butyldimethylsilyloxy)-6,7-secoabieta-8,11,13-trien-6,7-dial (28)

Ozone gas was passed through a solution of **27** (3.49 g, 8.77 mmol) in CH_2Cl_2 (200 ml) at −78 °C until pale blue color of the solution maintained. After the reaction, excess amount of ozone was released by bubbling nitrogen

gas. To the reaction mixture, a solution of triphenylphosphine (4.60 g, 17.5 mmol) in CH_2Cl_2 (20 ml) was added and the mixture was stirred for 1 h at −78 °C and for another 1 h at room temperature. The organic solvent of the reaction mixture was evaporated and the residue was purified by silica gel column chromatography (hexane/ethyl acetate = 20:1) to afford **28** (3.37 g, 89%) as a colorless oil; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 0.30, 0.68, 1.00 (each 3H, s), 1.04 (9H, s), 1.22, 1.23 (3H, d, J = 7.0 Hz), 1.48 (3H, s), 1.51–1.57 (1H, m), 1.62–1.74 (2H, m), 1.78–1.82 (2H, m), 2.27–2.34 (1H, m), 3.13 (1H, d, J = 4.0 Hz), 3.26 (1H, septet, J = 7.0 Hz), 6.94, 7.83 (each 1H, s), 9.88 (1H, d, J = 4.0 Hz), 10.50 (1H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ: −4.0 (2C), 18.3, 19.3, 22.45, 22.53, 25.7 (3C), 26.3, 27.7, 28.5, 30.3, 33.5, 37.3, 37.4, 40.4, 64.6, 117.3, 128.2, 134.0, 137.0, 150.3, 157.7, 191.5, 205.5; IR ($CHCl_3$): 2962, 2932, 1717, 1672, 1595, 1497, 1259 cm^{-1}; MS (70 eV) m/z: 430 (M^+), 401, 373, 345, 317, 249, 221, 179; HRMS calcd for $C_{26}H_{42}O_3Si$ (M^+): 430.2903, found: 430.2905.

4.26. 12-Hydroxy-6,7-secoabieta-8,11,13-trien-6,7-dial (2)

4.26.1. Preparation from 26. Potassium carbonate (31 mg, 0.22 mmol) was added to a solution of **26** (40 mg, 0.11 mmol) in methanol (1 ml) and the mixture was stirred for 5 h at room temperature. After the reaction, the reaction mixture was diluted with water. The aqueous solution was neutralized with 1 M hydrochloric acid and extracted with ethyl acetate. The organic layer was washed with a saturated aqueous solution of sodium chloride, dried over $MgSO_4$, and condensed in vacuo. The residue was purified by preparative TLC (hexane/ethyl acetate = 4:1) to afford **2** (27.0 mg, 77%) as colorless crystals.

4.26.2. Preparation from 28. *tert*-Butylammonium fluoride (1.0 M in THF, 0.25 ml, 0.25 mmol) was added to a solution of **28** (90 mg, 0.21 mmol) in THF (2 ml) at 0 °C and the mixture was stirred for 30 min at room temperature. After the reaction, the reaction mixture was poured into a saturated aqueous solution of ammonium chloride and extracted with ethyl acetate. The organic layer was washed with a saturated aqueous solution of sodium chloride, dried over $MgSO_4$, and condensed in vacuo. The residue was purified by silica gel column chromatography (hexane/ethyl acetate = 4:1) to afford **2** (60 mg, 90%) as colorless crystals; mp: 195–197 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 0.70, 1.01 (each 3H, s), 1.26. 1.27 (each 3H, d, J = 6.9 Hz), 1.50 (3H, s), 1.50–1.83 (5H, m), 2.28–2.34 (1H, m), 3.13 (1H, d, J = 3.8 Hz), 3.18 (1H, septet, J = 6.9 Hz), 6.34 (1H, br s), 6.96, 7.84 (each 1H, s), 9.86 (1H, d, J = 3.8 Hz), 10.47 (1H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 19.3, 22.20, 22.23, 26.7, 27.4, 28.1, 30.6, 33.5, 37.4, 37.6, 40.4, 64.6, 114.8, 127.9, 132.4, 134.6, 151.1, 157.9, 191.6, 206.2; IR (KBr): 3352, 2959, 2934, 1701, 1661, 1593, 1570, 1261, 1173 cm^{-1}; MS (70 eV) m/z: 316 (M^+), 298, 283, 255, 203, 171; HRMS calcd for $C_{20}H_{28}O_3$ (M^+): 316.2038, found: 316.2039; Anal. Calcd for $C_{20}H_{28}O_3$: C, 75.91; H, 8.92. Found: C, 75.68; H, 8.85.

4.27. DL-Standishinal (1)

(+)-Camphorsulfonic acid (17 mg, 0.073 mmol) was added to a solution of **2** (19 mg, 0.061 mmol) in CH_2Cl_2 (3 ml) and the mixture was stirred for 8 h at room temperature. After the reaction, the reaction mixture was extracted with ethyl acetate and the organic layer was successively washed with a saturated aqueous solution of sodium bicarbonate and a saturated aqueous solution of sodium chloride, dried over $MgSO_4$, and condensed in vacuo. The residue was purified by silica gel preparative TLC (hexane/ethyl acetate = 4:1) to afford DL-**1** (16 mg, 84%) as colorless crystals; mp: 187–189 °C (hexane/chloroform); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.17, 1.19 (each 3H, s), 1.24, 1.25 (each 3H, d, J = 6.9 Hz), 1.26 (3H, s), 1.21–1.26 (1H, m), 1.51–1.55 (1H, m), 1.82 (1H, d, J = 10.2 Hz), 1.61–1.91 (3H, m), 2.10 (1H, d, J = 8.1 Hz), 2.44–2.48 (1H, m), 3.27 (1H, septet, J = 6.9 Hz), 5.40 (1H, dd, J = 10.2, 8.1 Hz), 7.69 (1H, s), 8.26 (1H, s), 10.21 (1H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 19.9, 22.0, 22.3, 22.4, 23.9, 26.5, 33.4, 38.4, 41.0, 46.7, 67.2, 75.5, 124.5, 127.2, 128.5, 134.5, 153.9, 157.3, 189.9; IR (KBr): 3450, 3252, 2962, 2932, 2870, 1664, 1612, 1580, 1479, 1466, 1261, 1171, 1103 cm^{-1}; MS (70 eV) m/z: 316 (M^+), 298, 283, 269, 255, 229, 213, 199, 171; HRMS calcd for $C_{20}H_{28}O_3$ (M^+): 316.2038, found: 316.2039; Anal. Calcd for $C_{20}H_{28}O_3$: C, 75.91; H, 8.92. Found: C, 76.03; H, 8.83.

4.28. Reduction of 21

A solution of compound **21** (51.3 mg, 0.163 mmol) in tetrahydrofuran (1.0 ml) was added to a suspension of lithium aluminum hydride (1.24 mg, 0.327 mmol) in tetrahydrofuran (1.0 ml) and the reaction mixture was stirred for 1 h at 0 °C and for another 1 h at room temperature. The reaction was quenched by adding a saturated aqueous solution of sodium sulfate and the mixture was filtered through Celite®. The filtrate was extracted with diethyl ether and the organic layer was washed with a saturated aqueous solution of sodium chloride, dried over sodium sulfate, and condensed in vacuo. The residue was purified by preparative silica gel thin layer chromatography (hexane/ethyl acetate = 5:1) to afford **29** (43.3 mg, 84%). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 0.47, 1.00 (each 3H, s), 1.21 (6H, d, J = 7.0 Hz), 1.24 (3H, s), 2.10 (1H, m), 2.54 (1H, ddd, J = 6.6, 7.9, 14.3 Hz), 3.27 (1H, septet, J = 7.0 Hz), 3.82 (3H, s, OMe), 4.81 (1H, br q, J = 6.6 Hz), 6.72, 7.37 (each 1H, s).

4.29. Dehydration of 29

A solution of compound **29** (33.1 mg, 0.105 mmol) and *p*-toluenesulfonic acid (2.0 mg) in benzene (1.0 ml) was stirred under refluxed condition for 1 h. The reaction mixture was then poured into a saturated aqueous solution of sodium bicarbonate and the mixture was extracted with ethyl acetate. The organic layer was washed with a saturated aqueous solution of sodium chloride, dried

over sodium sulfate, and condensed in vacuo. The residue was purified by preparative silica gel thin layer chromatography (hexane) to afford **30** (29.0 mg, 93%) as a colorless oil; ^{1}H NMR, (400 MHz, $CDCl_3$) δ: 0.35, 0.88, 1.09 (each 3H, s), 1.192, 1.194 (each 3H, d, J = 6.9 Hz), 1.78 (1H, d, J = 6.3 Hz), 2.40 (1H, br d, J = 12.1 Hz), 3.24 (1H, septet, J = 6.9 Hz), 3.84 (3H, s, OMe), 5.84 (1H, dd, J = 6.3, 9.7 Hz), 6.45 (1H, J = 9.7 Hz), 6.75, 6.83 (each 1H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 19.3, 20.5, 22.7, 22.8, 26.4, 31.2, 33.4, 35.1, 35.5, 37.0, 42.1, 52.5, 55.7, 105.5, 124.7, 125.9, 127.3, 128.1, 133.5, 141.5, 156.3; IR ($CHCl_3$) 3007, 2961, 2936, 2864, 1499, 1464, 1248, 1057 cm^{-1}; MS (70 eV) *m*/*z*: 298 (M^+), 298, 283, 241, 227, 216, 199, 173; HRMS calcd for $C_{21}H_{30}O$ (M^+): 298.2297, found: 298.2290.

4.30. Conversion of 30 to 31

2.71 M solution of *n*-butyllithium in hexane (1.92 ml, 5.21 mmol) was added to a solution of dodecanethiol (1.25 ml, 5.21 mmol) in HMPA (8.0 ml) and the mixture was stirred for 1 h at 0 °C. The mixture was then added to compound **29** (155.6 mg, 0.521 mmol) at room temperature and the reaction mixture was stirred for 1.5 h at 100 °C. The reaction was quenched by adding a saturated aqueous solution of ammonium chloride and 1 M hydrochloric acid and the mixture was extracted with diethyl ether. The organic layer was washed with a saturated aqueous solution of sodium chloride, dried over sodium sulfate, and condensed in vacuo. The residue was purified by preparative silica gel column chromatography (hexane/ethyl acetate = 25:1) to afford demethylated derivative (146.1 mg, 99%), to which the solution in dimethylformamide (5.0 ml), *tert*-butyldimethylsilyl chloride (154.8 mg, 1.03 mmol), and imidazole (139.9 mg, 2.05 mmol) were successively added. After stirring the reaction mixture for 10 h at room temperature, a saturated aqueous solution of ammonium chloride was added. The mixture was extracted with diethyl ether and the organic layer was washed with a saturated aqueous solution of sodium chloride, dried over sodium sulfate, and condensed in vacuo. The residue was purified by preparative silica gel column chromatography (hexane/ethyl acetate = 50:1) to afford **31** (188.1 mg, 92%) as a colorless oil; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 0.21, 0.24, 0.35, 0.88 (each 3H, s), 1.02 (9H, s), 1.06 (3H, s), 1.17, 1.18 (each 3H, d, J = 6.9 Hz), 1.77 (1H, d, J = 6.2 Hz), 2.30 (1H, br d, J = 12.3 Hz), 3.23 (1H, septet, J = 6.9 Hz), 5.83 (1H, dd, J = 6.7, 9.7 Hz), 6.45 (1H, d, J = 9.7 Hz), 6.67, 6.82 (each 1H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 18.3, 19.1, 20.5, 22.9, 23.0, 25.9, 26.2, 31.3, 33.4, 35.1, 35.5, 36.6, 41.9, 52.4, 113.3, 124.6, 126.1, 127.8, 128.1, 135.3, 141.3, 152.1; IR ($CHCl_3$): 3032, 2961, 2932, 2862, 1495, 1271, 1236, 1200 cm^{-1}; MS (70 eV) *m*/*z*: 398 (M^+), 383, 316; HRMS calcd for $C_{26}H_{42}OSi$ (M^+): 398.3005, found: 398.3001.

4.31. Ozonolysis of 31

Ozone gas was passed through a solution of **31** (188.1 mg, 0.472 mmol) in CH_2Cl_2 (20 ml) at −78 °C until pale blue color of the solution maintained. After the reaction, excess amount of ozone was released by bubbling nitrogen gas. To the reaction mixture, a solution of triphenylphosphine in CH_2Cl_2 was added and the mixture was stirred for 1 h at −78 °C and for another 1 h at room temperature. The organic solvent of the reaction mixture was evaporated and the residue was purified by silica gel column chromatography (hexane/ethyl acetate = 15:1) to afford **32** (170.1 mg, 84%). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 0.276, 0.278 (each 3H, s), 0.89 (3H, s), 1.02 (9H, s), 1.18, 1.20 (each 3H, d, J = 6.9 Hz), 1.28, 1.67 (each 3H, s), 2.28 (1H, dt, J = 4.2, 12.5 Hz), 3.11 (1H, d, J = 5.6 Hz), 3.20 (1H, septet, J = 6.9 Hz), 6.81, 7.79 (each 1H, s), 9.36 (1H, d, J = 5.6 Hz), 10.43 (1H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 18.3, 19.0, 22.2, 22.6, 25.7, 26.3, 28.7, 30.2, 31.4, 34.0, 35.1, 36.1, 40.2, 64.3, 116.1, 128.8, 136.9, 149.7, 157.8, 191.6, 204.2; IR ($CHCl_3$): 3032, 2961, 2932, 2862, 1715, 1595, 1267, 1258, 1232, 1221, 1204 cm^{-1}; MS (70 eV) *m*/*z*: 430 (M^+), 401, 387, 373, 300, 285, 243; HRMS calcd for $C_{26}H_{42}O_3Si$ (M^+): 430.2903, found: 430.2897.

4.32. Desilylation of 32

A solution of TBAF in tetrahydrofuran was added to a solution of **32** (170.1 mg, 0.395 mmol) in tetrahydrofuran (4.0 ml) and the mixture was stirred for 30 min at room temperature. A saturated aqueous solution of ammonium chloride was added to the reaction mixture, which was extracted with ethyl acetate. The organic layer was washed with a saturated aqueous solution of sodium chloride, dried over sodium sulfate, and condensed in vacuo. The residue was purified by preparative silica gel column chromatography (hexane/ethyl acetate = 4:1) to afford **33** (113.1 mg, 91%). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 0.90 (3H, s), 1.23, 1.25 (each 3H, d, J = 6.9 Hz), 1.28, 1.67 (each 3H, s), 2.33 (1H, dt, J = 4.0, 12.6 Hz), 3.11 (1H, septet, J = 6.9 Hz), 3.14 (1H, d, J = 5.6 Hz), 5.81 (1H, br d, J = 10.4 Hz), 6.82, 7.80 (each 1H, s), 9.39 (1H, d, J = 5.6 Hz), 10.42 (1H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 18.9, 22.0, 22.3, 26.7, 28.6, 30.2, 31.2, 34.1, 35.1, 36.1, 40.2, 64.3, 113.3, 128.7, 132.2, 133.6, 150.5, 157.8, 191.5, 205.1; IR (KBr): 3140, 2957, 2870, 1717, 1647, 1585, 1570, 1269, 1171 cm^{-1}; MS (70 eV) *m*/*z*: 316 (M^+), 298, 283, 255, 242, 229, 109; HRMS calcd for $C_{20}H_{28}O_3$ (M^+): 316.2038, found: 316.2036; Anal. Calcd for $C_{20}H_{28}O_3$: C, 75.91; H, 8.92. Found: C, 75.91; H, 8.75.

4.33. Aromatase inhibitory assay

Aromatase inhibitory assays were carried out using the CYP19/ Methoxy-4-trifluoromethylcoumarin (MFC) High Throughput Inhibitory Screening Kit (BD Gentest, Woburn, MA, USA). MFC, the fluorometric substrate, is rapidly converted by human recombinant aromatase expressed in insect cells using the baculovirus systems to the highly fluorescent product. Positive control inhibitors for aromatase, ketoconazole and letrozole, were purchased from Wako Pure Chemical Industries, Ltd (Osaka, Japan) and LKT Laboratories, Inc. (St. Paul, MN), respectively.

The NADPH-cofactor buffer for dilution of compounds and enzyme–substrate mixture were prepared according to the manufacturer's instruments. The test compounds and positive control inhibitors (dissolved in acetone) were diluted serially in 1:3 ratio by the NADPH-cofactor buffer in 96-well microplates. The reaction was initiated by the addition of prewarmed enzyme–substrate mixture and incubated for 30 min at 37 °C. Changes in fluorescence value were measured with a multifunctional microplate detection system ULTRA with suitable 409 nm excitation filters and 530 nm emission interference (Tecan Japan Co. Ltd, Kanagawa, Japan).

The result obtained under the condition (Table 3) is also viewed in Figure 2.

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