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Synthesis and biological evaluation of γ -alkylidenebutenolides isolated from *Melodorum fruticosum*: the role of the propylidene-type side chain structure on anti-melanogenic activity†

Katsuki Takashima,^a Yoshiaki Manse,^b Riko Suzuki,^a Nanami Masuda,^a Yuki Fukuda,^a Risa Fukuda,^a Shinsuke Marumoto,^c Fumihiko Ishikawa,^a Toshio Morikawa^{b*} and Genzoh Tanabe^{b*}

The first structure–activity relationship study on γ -alkylidenebutenolides [(4Z)- and (4E)-6,7-dihydroxyhepta-2,4-dien-4-olide 6-monobenzoate] (4Z, **3** and 4E, **4**) and 7-monobenzoate (4Z, **1** and 4E, **2**) which are potent melanogenesis inhibitors (IC₅₀ = 0.3–2.9 μ M) isolated from the medicinal plant *Melodorum fruticosum*, was carried out using the related analogous (**7–21**). The inhibitory activities of the (4Z)- and (4E)-6,7-dideoxylated compounds (**7**) completely disappeared, while those of the 6-oxo-7-hydroxy-type compounds (4Z, **20** and 4E, **21**) were significantly attenuated compared with those of **1–4**. By contrast, the 6,7-dihydroxylated compounds (4Z, **8** and 4E, **9**; IC₅₀ = 5.8–1.5 μ M) showed inhibitory activities similar to those of **1–4**, suggesting that the two hydroxyl groups at positions C6 and C7 on the side chain play an essential role in the onset of potent inhibitory activity. The inhibitory activities of the 6,7-diacylated analogues (**10–19**, acyl group = Ac, Piv, or Bz; IC₅₀ = 0.7–3.3 μ M) were also nearly identical to those of the 6- or 7-monobenzoates (**1**, **3**, and **4**), regardless of the geometry of the double bond. However, acylation of the strongest inhibitor (4E, **2**; IC₅₀ = 0.3 μ M) at position C6, reduced the activity by approximately 1/3 to 1/5. This result suggests the importance of the cooperative role of the acyl moiety at position C7 and the hydroxyl group at position C6 in the *E*-configured double bond. The total synthesis of the natural products melodorinone A (**20**) and melodorinone B (**21**) was also achieved during the synthesis of the γ -alkylidenebutenolide analogues.

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Introduction

The ring system of 2,5-dihydrofuran-2-one [furan-2(5*H*)-one], also known as $\Delta_{\alpha,\beta}$ -butenolide, is an important core shared by a number of natural products.¹ A large number of $\Delta_{\alpha,\beta}$ -butenolide derivatives with an exocyclic double bond at the γ -position have been found in nature. These derivatives are called γ -alkylidenebutenolides, and many of them have been reported to exhibit beneficial biological activities. For example, the simplest γ -alkylidenebutenolide, protoanemonin (**A**), exhibits various biological activities, such as cytotoxic, antibacterial,

and antifungal activities.^{2–7} Bioactive γ -alkylidenebutenolides with more complex structures such as enhydrolide (**B**),⁸ piptocarfine A (**C**),⁹ and EBC-329 (**D**),¹⁰ exhibit antibacterial, cytotoxic, and antiproliferative activities, respectively, and cryptonconatone I (**E**),¹¹ and aspergone B (**F**),¹² inhibit nitric oxide production and α -glucosidase activity, respectively (Fig. 1). Therefore, these natural products have attracted considerable attention as important sources of new therapeutic and physiological agents. In this context, the total synthesis^{13–17} and development of synthetic methods^{18–20} for these compounds have been actively investigated by many organic and bio-organic chemists.

Melodorinol (**1**), isomelodorinol (**2**), fruticosinol (**3**), and isofruticosinol (**4**) are isolated from the medicinal plant *Melodorum fruticosum* (also known as devil's tree or white cheesewood), which is endemic to Vietnam, Laos, Cambodia, and Thailand,²¹ and have a hepta-2,4-dien-4-olide (also called γ -propylidene- $\Delta_{\alpha,\beta}$ -butenolide) structure belonging to the γ -alkylidenebutenolide family. The chiral HPLC analysis of optically pure (*S*)- and (*R*)-butenolides [(*S*)-**1**-(*S*)-**4** and (*R*)-**1**-

^aFaculty of Pharmacy, Kindai University, 3-4-1 Kowakae, Higashi-osaka, Osaka 577-8502, Japan

^bPharmaceutical Research and Technology Institute, Kindai University, 3-4-1 Kowakae, Higashi-osaka, Osaka 577-8502, Japan

^cJoint Research Centre, Kindai University, 3-4-1 Kowakae, Higashi-osaka, Osaka 577-8502, Japan

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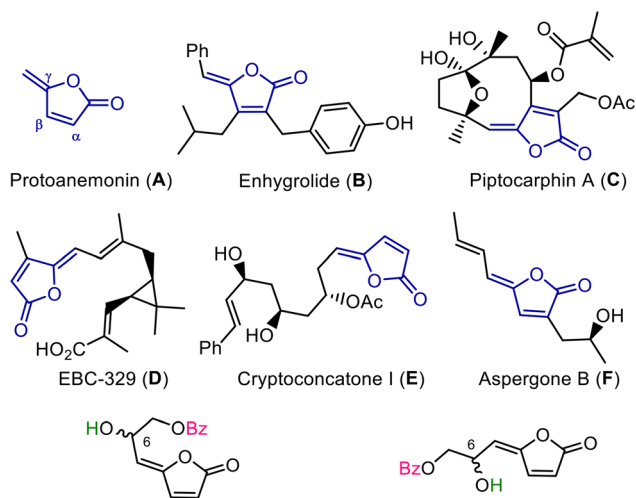


Fig. 1 Chemical structures of representative natural γ -alkylidenebutenolides.

(*R*-4], independently synthesised in our laboratory from *D*- and *L*-ribose, respectively, revealed that all isolates (1–4) from this plant are partially racemic mixtures predominantly containing the *S*-isomer (~66% ee).²² Additionally, as part of our ongoing search for bioactive constituents from folk medicinal plants, we demonstrated that both enantiomers of butenolides 1–4 are potent melanogenesis inhibitors, regardless of their stereochemistry at position C6. (*R*-2 and *S*-2 (IC_{50} = 0.29–0.39 μ M) are particularly active compounds, exhibiting inhibitory activities that are 400–500 times more potent than that of arbutin (5, IC_{50} = 174 μ M), a standard skin-lightening agent with potent melanin production inhibitory activity (Fig. 2). Therefore, these γ -alkylidenebutenolides can be considered ideal seeds for melanogenesis inhibitors.

Melanin is a pigment that determines the colour of human hair, eyes, and skin. Normal melanin pigmentation is involved in many beneficial functions, such as UV shielding, photocarcinogenesis inhibition, and vitamin D3 biosynthesis.²³ Abnormal melanin pigmentation causes serious dermatologi-

cal issues, such as senile lentigines, freckles, melasma, and other melanin hyperpigmentations. Therefore, controlling melanogenesis may be an important approach for treating pigmentation-related disorders. Some phenomena associated with melanin accumulation may also be correlated with the development of Parkinson's disease, Alzheimer's disease, and melanoma.^{24,25} Thus, melanogenesis regulators are required for the chemical-based functional analysis of central nervous system diseases and malignancies. However, synthetic access to melanogenesis inhibitors and our understanding of their mode of action are currently limited.

As a continuation of our research project, we designed and synthesised relevant γ -alkylidenebutenolide-based analogues and evaluated their anti-melanogenic inhibitory activity to gain new insights into the design of better melanogenesis inhibitors. The synthesised materials were (a) compounds 6 and 7, which are the main carbon skeletons of natural products 1–4, (b) diols (*S*)-8 and (*S*)-9, (c) both enantiomers of diesters (*S*)-10–(*S*)-19 and (*R*)-10–(*R*)-19, and (d) ketones 20 and 21. This study is the first to examine the structure–activity relationship underlying the inhibition of melanogenesis based on the structure of γ -alkylidenebutenolides originating from *M. fruticosum*. Furthermore, we also report the first synthesis of ketones 20 (melodrinone A) and 21 (melodrinone B), which are cytotoxic natural products also isolated from *M. fruticosum* (Fig. 3).

Results and discussion

Compounds 6 and 7 were synthesised as shown in Scheme 1. According to the method reported by Jefford *et al.*, 2-(trimethylsilyloxy)furan (22) was alkylated with 1-iodopropane in the presence of silver trifluoromethanesulfonate to afford 6,²⁶ albeit in low yield. The TMSOTf-induced aldol reaction of the furan derivative 22 with propanal afforded 23 as a diastereomeric mixture (*erythro/threo* ratio = *ca.* 1/4). The subsequent E1cB elimination of the hydroxyl group of 23 with acetic anhydride (Ac_2O) under basic conditions afforded 7²⁷ as a mixture of *E*- and *Z*-isomers (*E/Z* ratio = *ca.* 2/3).

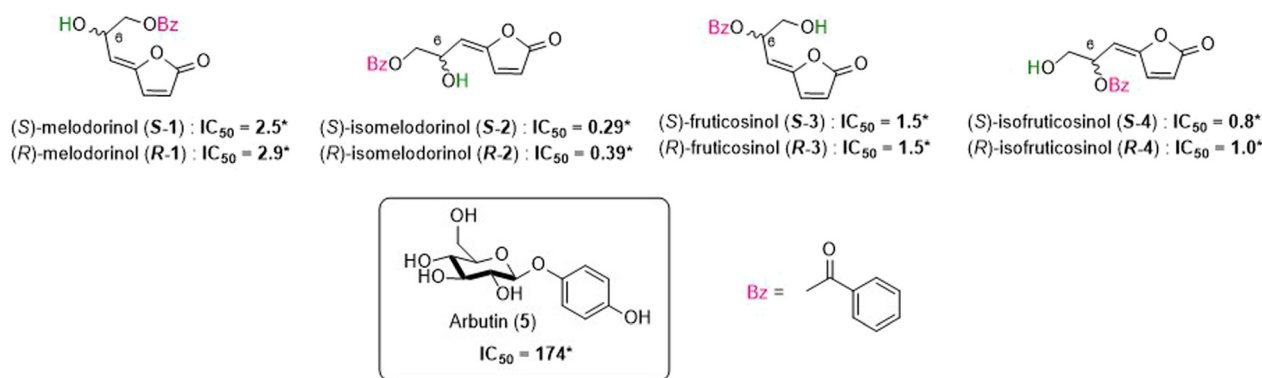


Fig. 2 Chemical structures of γ -alkylidenebutenolides isolated from *Melodorum fruticosum* and arbutin, a reference standard for skin-whitening. *Melanogenesis inhibitory activity (IC_{50} value, μ M).



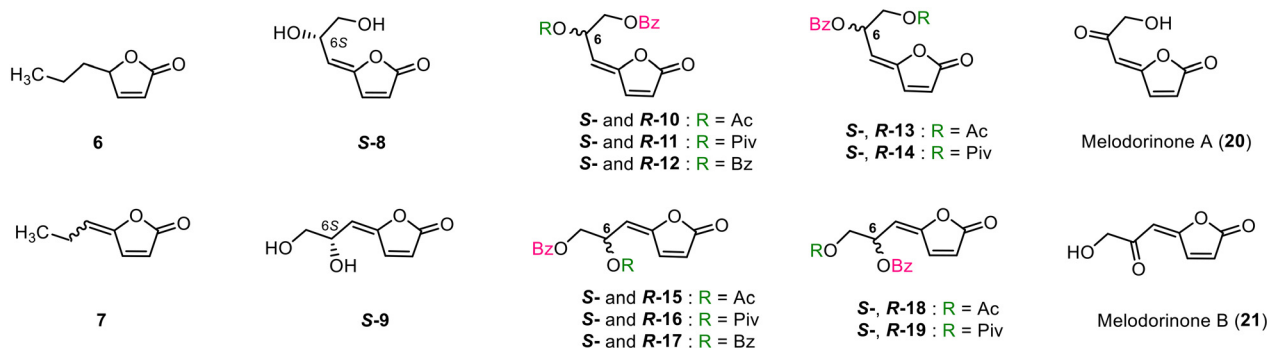
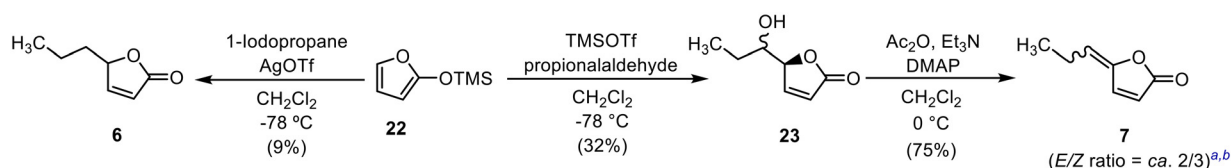


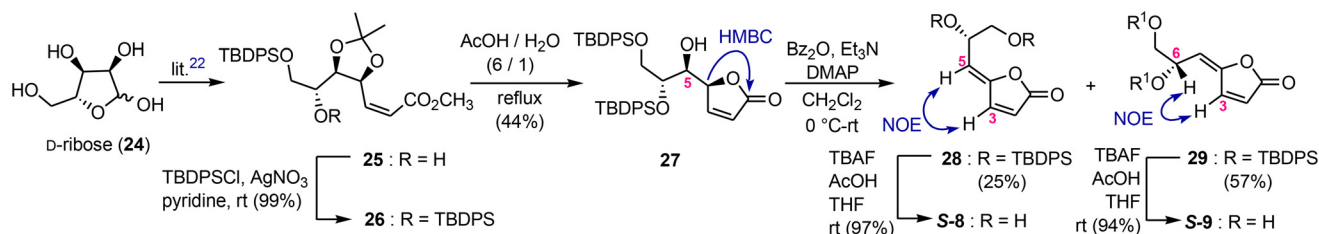
Fig. 3 Designed analogues 6–21.

Scheme 1 Synthesis of analogues 6 and 7: ^a the stereochemistry of the *E*- and *Z*-isomers was confirmed by NOESY experiments; ^b the *E/Z* ratio was estimated by ¹H NMR analysis.

Next, the dihydroxylated compounds (**S**)-**8** and (**S**)-**9** were synthesised from a known α,β -unsaturated ester **25** prepared from *D*-ribose (**24**) according to the procedure²² used to synthesise natural products **3** and **4** (Scheme 2). The secondary hydroxyl group of **25** was protected with a TBDPS group to quantitatively afford silyl ether **26**, which was then heated in aqueous acetic acid at 100 °C to afford the corresponding butenolide **27** in 44% yield. The IR spectrum of **27** showed two characteristic absorption bands at higher wavenumbers (1786 and 1747 cm^{-1}) than the single band of its precursor **26** (1720 cm^{-1}), thus supporting the formation of the α -unsubstituted $\Delta_{\alpha,\beta}$ -butenolide ring. The HMBC correlation between the signals corresponding to H-4 and C-1, as shown in Scheme 2, confirmed these findings. The ¹H NMR spectrum of **27** showed two signals at δ_{H} 6.03 and 7.21, which were attributed to the α and β protons of the $\Delta_{\alpha,\beta}$ -butenolide ring, respectively, with a J_{2-3} value of 5.8 Hz, indicating a *cis*-configuration of the olefin moiety. Subsequently, **27** was subjected to an E1cb elimination reaction by treatment with benzoic anhydride under basic conditions to afford (*Z*)-

γ -alkylidenebutenolide **28** and its *E*-isomer **29** in 25% and 57% yields, respectively. The stereochemistries of the newly introduced *exo*-double bonds in **28** and **29** were determined to be *cis* and *trans*, respectively, based on nuclear Overhauser effect spectroscopy (NOESY) experiments, as shown in Scheme 2. Finally, desilylation of **28** and **29** using tetrabutylammonium fluoride (TBAF) in the presence of excess acetic acid (3 equiv.) in THF afforded **S**-**8** and **S**-**9** in 97% and 94% yields, respectively. **S**-**8** and **S**-**9** showed the same NOESY correlations as their corresponding materials (**28** and **29**), supporting the stereochemistries of the *exo*-double bonds depicted in Scheme 2.

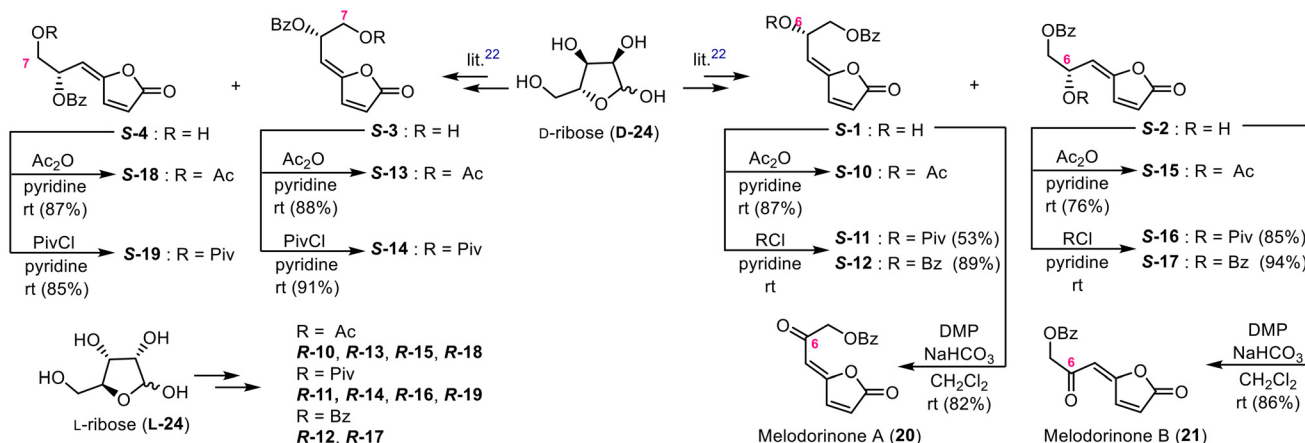
(*S*)-Melodorinol [(**S**)-**1**], (*S*)-isomelodorinol [(**S**)-**2**], (*S*)-fruticosinol [(**S**)-**3**], and (*S*)-isofruticosinol [(**S**)-**4**] were synthesised from *D*-ribose according to our method²² and then further converted into the corresponding diesters [(**S**)-**10**–(**S**)-**19**] in good yields by reaction with acylating reagents, such as Ac₂O, pivaloyl chloride (PivCl), and benzoyl chloride (BzCl). The enantiomers [(**R**)-**1**–(**R**)-**4**] of (**S**)-**1**–(**S**)-**4** were similarly synthesised from *L*-ribose (**L**-**24**) and converted into the corresponding acetates [(**R**)-**13** and (**R**)-**18**] and pivaloates [(**R**)-**14** and (**R**)-**19**]. Finally,

Scheme 2 Synthesis of dihydroxylated analogues (**8** and **9**).

the secondary hydroxyl groups in (*S*)-**1** and (*S*)-**2** were oxidised with Dess–Martin periodinane to give **20** and **21** in 82 and 86% yields, respectively. The ¹H and ¹³C NMR properties of synthesised **20** and **21** were in good agreement with those of natural melodorinone A (**20**)²⁸ and melodorinone B (**21**) (Scheme 3).²⁸

With all the target compounds in hand, we examined their melanogenesis inhibitory activities in theophylline-stimulated B16 melanoma 4A5 cells (Table 1 and ESI Tables S3 and S4†). First, the inhibitory activities of Δ_{αβ}-butenolides **6** and **7** were evaluated to determine the correlation between the carbon skeletons of **1–4** and their activity. Compound **6**, a potential Michael acceptor, showed no inhibitory activity even at concentrations as high as 100 μM. Similarly, γ-propylidene-Δ_{αβ}-bute-

nolide (**7**), the main carbon skeleton of **1–4**, exhibited no inhibitory activity, suggesting that oxygen functionalization of the side chain, as observed in the structures of **1–4**, is required for the onset of strong inhibitory activity (entries 17 and 18 vs. 1, 2, 19, and 20). Bioassays of the diols (*S*)-**8** and (*S*)-**9** demonstrated that they were both significantly more potent inhibitors than **7**, a nonhydroxylated butenoide, with IC₅₀ values of 5.8 and 1.5 μM, respectively (entries 3 and 22 vs. 18). These results confirm the validity of our prediction of the importance of oxygen functionalisation. Next, the activity of the diol *S*-**8**, which has a *Z*-configured double bond, was compared with those of the corresponding mono-*O*-benzoates [(*S*)-**1** and (*S*)-**3**]. As shown in Table 1, *O*-benzylation of the hydroxyl group at either position 6 or 7 of the side chain led to slightly increased



Scheme 3 Synthesis of diesters [(*S*)-**10**–(*S*)-**19** and (*R*)-**10**–(*R*)-**19**] and total synthesis of melodorinones (**20** and **21**).

Table 1 Melanogenesis inhibitory activity (IC₅₀, μM) of γ-alkylidenebutenolides (**1–4**) and related compounds (**8–21**)

Entry	Compound	R ¹	R ²	IC ₅₀ (μM)	Entry	Compound	R ¹	R ²	IC ₅₀ (μM)
1	<i>S</i> -Melodorinol (S - 1)	H	Bz	2.7, (lit. ²² 2.5)	19	<i>S</i> -Isomelodorinol (S - 2)	H	Bz	0.29 ²²
2	<i>S</i> -Fructosinol (S - 3)	Bz	H	1.5 ²²	20	<i>S</i> -Isofructosinol (S - 4)	Bz	H	0.80 ²²
3	S - 8	H	H	5.8	22	S - 9	H	H	1.5
4	S - 10	Ac	Bz	0.67	23	S - 15	Ac	Bz	1.4
5	S - 11	Piv	Bz	0.92	24	S - 16	Piv	Bz	0.88
6	S - 12	Bz	Bz	2.0	25	S - 17	Bz	Bz	1.5
7	S - 13	Bz	Ac	2.9	26	S - 18	Bz	Ac	0.89
8	S - 14	Bz	Piv	1.1	27	S - 19	Bz	Piv	2.0
9	<i>R</i> -Melodorinol (<i>R</i> - 1)	H	Bz	2.9 ²²	28	<i>R</i> -Isomelodorinol (<i>R</i> - 2)	Bz	Bz	0.39 ²²
10	<i>R</i> -Fructosinol (<i>R</i> - 3)	Bz	H	1.5 ²²	29	<i>R</i> -Isofructosinol (<i>R</i> - 4)	H	H	1.0 ²²
11	R - 10	Ac	Bz	1.0	30	R - 15	Ac	Bz	1.2
12	R - 11	Piv	Bz	0.82	31	R - 16	Piv	Bz	1.8
13	R - 12	Bz	Bz	3.3	32	R - 17	Bz	Bz	2.4
14	R - 13	Bz	Ac	3.3	33	R - 18	Bz	Ac	1.1
15	R - 14	Bz	Piv	1.2	34	R - 19	Bz	Piv	0.89
16	Melodorinone A (20)			71	35	Melodorinone B (21)			33
17	6			>100	36	Arbutin (5)			174 ²²
18	7			>100					



inhibitory activity (entries 3 vs. 1 and 2). A comparison of the activities of the *E*-isomer [(*S*)-9] of (*S*)-8 and its corresponding benzoates [(*S*)-2 and (*S*)-4] revealed that *O*-benzylation at position C6 did not lead to an increase in inhibitory activity; interestingly, however, *O*-benzylation at position C7 strengthened this activity by one order of magnitude (entries 22 vs. 19 and 20).

The above results motivated us to evaluate the effect of introducing a benzoyl group to the side chains of potent inhibitors [(*S*)-1–(*S*)-4]. In addition to the benzoyl group, the substitution effects of the relatively polar Ac group and bulky Piv group were also evaluated. Among the five *Z*-type diesters [(*S*)-10–(*S*)-14], (*S*)-10 exhibited a slight increase in inhibitory activity (entry 4, $IC_{50} = 0.67 \mu\text{M}$), but the potencies of the other compounds [(*S*)-11–(*S*)-14] remained nearly identical (entries 5–8, $IC_{50} = 2.9\text{--}0.92 \mu\text{M}$) to those of their parent compounds [(*S*)-1 and (*S*)-3]. The corresponding *E*-type diesters [(*S*)-15–(*S*)-19] also showed good inhibitory activities, with IC_{50} values ranging from 2.0 to 0.89 μM (entries 23–27). Thus, diacylation of the side chain did not lead to enhancements in inhibitory activity. On the contrary, the inhibitory activities of (*S*)-15, (*S*)-16 and (*S*)-17 were unexpectedly reduced to one-third to one-fifth that of the most potent inhibitor [(*S*)-2], indicating that acylation of (*S*)-4 at position C7 is not a suitable structural modification. Additionally, the 10 *6R*-diesters [(*R*)-10–(*R*)-14 and (*R*)-15–(*R*)-19] showed inhibitory activities similar to those of the corresponding *6S*-diesters [(*S*)-10–(*S*)-14 and (*S*)-15–(*S*)-19]. The enantiomers of the natural products (1–4) showed the same tendency as the diesters, suggesting that the stereoconfiguration of C6, regardless whether the substituent is –OH or –OCOR, plays a minor role in its activity. Melodrinone A (20) and melodrinone B (21) exhibited melanogenesis inhibitory activity with IC_{50} values of 71 and 33 μM , respectively. The IC_{50} of 20 was one order of magnitude lower than that of the corresponding alcohols [(*S*)-1 and (*R*)-1] while that of 21 was two orders of magnitude lower than that of the corresponding alcohols [(*S*)-2 and (*R*)-2] (entries 1 and 9 vs. 16, entries 19 and 28 vs. 35), suggesting that the presence of an oxygen atom with an sp^3 hybridisation orbital at position C6 is an important factor in the onset of potent inhibitory activity for this group of compounds.

Conclusions

In summary, we synthesised 26 analogues of natural γ -alkylidenebutenolides (1–4) and achieved the first total synthesis of melodrinone A (20) and melodrinone B (21). Evaluation of the inhibitory activity of all synthetics suggested that γ -alkylidenebutenolides require an *E*-configured side chain to express strong melanogenesis inhibitory activity. The importance of the cooperative role of the acyl moiety at position C7 and the oxygen atom with an sp^3 hybrid orbital at position C6 was also highlighted. We confirmed that the stereochemistry of the C6 centre bearing the oxygen functional group did not contribute to the onset of potent inhibitory

activity. Further studies on the structure–activity relationships of γ -alkylidenebutenolides with potential melanogenesis inhibitory activity are ongoing in our laboratory.

Experimental section

Chemistry

General. Mps were determined on a hot-stage melting point apparatus and are uncorrected. IR spectra were measured on a FT-IR spectrophotometer. NMR spectra were recorded on a FT-NMR spectrometers (^1H , 500, 600, or 800 MHz; ^{13}C , 125, 150, or 200 MHz). Chemical shifts (δ) and coupling constants (J) are given in ppm and Hz, respectively. Tetramethylsilane (TMS) was used as an internal standard for ^1H NMR measurements in CDCl_3 , whereas ^{13}C NMR measurements utilised the solvent signal (77.0 ppm) of CDCl_3 for this purpose. When CD_3OD was used for the measurement of ^1H and ^{13}C NMR spectra, solvents signal [δ_{H} 3.30 ppm and δ_{C} 49.0 ppm] were used as standards. 1D NMR peak assignments were confirmed by COSY, HSQC and HMBC spectra. High-resolution mass spectra were recorded on an orbitrap mass spectrometer (ESI). Optical rotations were determined with a digital polarimeter. All the organic extracts were dried over anhydrous Na_2SO_4 prior to evaporation. Column chromatography was performed over silica gel (45–106 μM).

5-Propylfuran-2(5H)-one (6). A mixture of 2-(trimethylsilyloxy) furan (22, 2.0 mL, 12.2 mmol), 1-iodopropane (1.54 mL, 15.9 mmol), silver trifluoroacetate (4.1 g, 15.9 mmol), and CH_2Cl_2 (20 mL) was stirred in the dark at -78°C for 5 h. The reaction mixture was filtered and the filtrate was condensed *in vacuo*. The residue was purified by column chromatography (*n*-hexane/ether = 10/1) to give the title compound²⁶ (6, 133 mg, 1.1 mmol, 9%) as a pale yellow oil. $^1\text{H-NMR}$ (500 MHz, CDCl_3) δ : 0.97 (3H, t, $J = 7.4$, $\text{CH}_2\text{CH}_2\text{CH}_3$), 1.46–1.52 (2H, m, $\text{CH}_2\text{CH}_2\text{CH}_3$), 1.61–1.69/1.71–1.79 (each 1H, m, $\text{CH}_2\text{CH}_2\text{CH}_3$), 5.06 (1H, dddd, $J = 7.4, 5.5, 1.8, 1.6$, H-5), 6.11 (1H, dd, $J = 5.6, 1.8$, H-4), 7.48 (1H, dd, $J = 5.6, 1.6$, H-2). $^{13}\text{C-NMR}$ (125 MHz, CDCl_3) δ : 13.7 ($\text{CH}_2\text{CH}_2\text{CH}_3$), 18.3 ($\text{CH}_2\text{CH}_2\text{CH}_3$), 35.1 ($\text{CH}_2\text{CH}_2\text{CH}_3$), 83.2 (C-5), 121.3 (C-3), 156.3 (C-4), 173.1 (C-2). HRMS (ESI) m/z : $[\text{M} + \text{Na}]^+$ calcd for $\text{C}_7\text{H}_{10}\text{O}_2\text{Na}$, 149.0573; found, 149.0574.

5-(1-Hydroxypropyl)furan-2(5H)-one (23). A mixture of 2-(trimethylsilyloxy)furan (29, 158 mg, 1.0 mmol), propionaldehyde (80 μL , 1.2 mmol), TMSOTf (40 μL , 0.20 mmol), and CH_2Cl_2 (3.0 mL) was stirred at -78°C for 3 h. The reaction was quenched with 10% hydrochloric acid (1.0 mL) and the resulting mixture was extracted with CH_2Cl_2 (5 mL \times 3). The extract was washed with brine (5 mL) and condensed *in vacuo*. The residue was purified by column chromatography (*n*-hexane/EtOAc = 10/1) to give the title compound 23²⁹ (45 mg, 0.32 mmol, 32%) as a *ca.* 1:4 mixture of *erythro* and *threo* isomers. The HRMS (ESI) spectrum of the mixture of *erythro*- and *threo*-23 showed two peaks due to $[\text{M} + \text{Na}]^+$ ions at m/z 165.0524 (for calcd for $\text{C}_7\text{H}_{10}\text{O}_3\text{Na}$, 165.0522).



Compound **erythro-23** (NMR data extracted from the spectrum of a mixture of **erythro-** and **threo-23**): $^1\text{H-NMR}$ (800 MHz, CDCl_3) δ : 1.06 (3H, t, $J = 7.6$, CHCH_2CH_3), 1.54–1.73 (2H, m, CHCH_2CH_3), 2.44 (1H, br s, OH), 3.79 (1H, br td, $J = ca. 8.5, 5.0, \text{H-6}$), 4.98 (1H, ddd, $J = 5.0, 2.1, 1.5, \text{H-5}$), 6.19 (2H, dd, $J = 6.0, 2.1, \text{H-3}$), 7.56 (1H, dd, $J = 6.0, 1.5, \text{H-4}$). $^{13}\text{C-NMR}$ (200 MHz, CDCl_3) δ : 9.9 (CHCH_2CH_3), 26.2 (CHCH_2CH_3), 72.8 (C-6), 85.8 (C-5), 122.7 (C-3), 153.7 (C-4), 173.0 (C-2).

Compound **threo-23** (NMR data extracted from the spectrum of a mixture of **erythro-** and **threo-23**): $^1\text{H-NMR}$ (800 MHz, CDCl_3) δ : 1.05 (3H, t, $J = 7.6$, CHCH_2CH_3), 1.60–1.70 (2H, m, CHCH_2CH_3), 2.35 (1H, br s, OH), 3.70 (1H, br td, $J = ca. 8.2, 4.6, \text{H-6}$), 5.02 (1H, ddd, 4.6, 2.1, 1.6, H-5), 6.19 (2H, dd, $J = 6.0, 2.1, \text{H-3}$), 7.48 (1H, dd, $J = 6.0, 1.6, \text{H-4}$). $^{13}\text{C-NMR}$ (200 MHz, CDCl_3) δ : 9.9 (CHCH_2CH_3), 26.3 (CHCH_2CH_3), 73.0 (C-6), 85.9 (C-5), 122.6 (C-3), 153.8 (C-4), 173.0 (C-2).

E1cb elimination reaction of erythro- and threo-23. A mixture of **erythro-** and **threo-23** (200 mg, 1.4 mmol), Et_3N (0.58 mL, 4.2 mmol), Ac_2O (0.27 mL, 2.8 mmol), DMAP (17 mg, 0.14 mmol), and CH_2Cl_2 (10 mL) was stirred at room temperature (rt) for 18 h. The reaction mixture was poured into cold water (20 mL) and extracted with CH_2Cl_2 (7 mL \times 3). The extract was successively washed with aqueous NaHCO_3 (7 mL) and brine (7 mL) and condensed *in vacuo*. The residue was purified by column chromatography (*n*-hexane/ether = 10/1) to give a *ca.* 2 : 3 mixture of *E*- and *Z*-5-propylidene-furan-2(5H)-one²⁷ [hepta-2,4-diene-4-olide (*E*- and *Z*-7, 132 mg, 1.06 mmol, 75%)]. The HRMS (ESI) spectrum of the mixture of *E*- and *Z*-7 showed two peaks due to $[\text{M} + \text{Na}]^+$ ions at m/z 125.0598 (calcd for $\text{C}_7\text{H}_9\text{O}_2$, 125.0597).

Compound **E-7** (NMR data extracted from the spectrum of a mixture of *E*- and *Z*-7): $^1\text{H-NMR}$ (800 MHz, CDCl_3) δ : 1.13 (3H, t, $J = 7.5, \text{H-7}$), 2.32 (2H, dq, $J = 8.3, 7.5, \text{H-6}$), 5.78 (1H, ddd-like, $J = ca. 8.3, 1.8, 0.8, \text{H-5}$), 6.19 (1H, dd, $J = 5.6, 1.8, \text{H-2}$), 7.64 (1H, dd, $J = 5.6, 0.8, \text{H-3}$); $^{13}\text{C-NMR}$ (200 MHz, CDCl_3) **E-20**: δ : 14.4 (C-7), 20.1 (C-6), 118.2 (C-5), 120.1 (C-2), 139.3 (C-3), 149.5 (C-4), 170.0 (C-1).

Compound **Z-7** (NMR data extracted from the spectrum of a mixture of *E*- and *Z*-7): $^1\text{H-NMR}$ (800 MHz, CDCl_3) δ : 1.11 (3H, t, $J = 7.6, \text{H-7}$), 2.43 (2H, quin, $J = 7.6, 0.4, \text{H-6}$), 5.29 (1H, br t-like, $J = ca. 7.6, \text{H-5}$), 6.14 (1H, dd, $J = 5.3, 0.6, \text{H-2}$), 7.33 (1H, br d-like, $J = ca. 5.3, 0.4, \text{H-3}$); **Z-20**: δ : 13.4 (C-7), 19.9 (C-6), 119.0 (C-2 and C-5), 143.6 (C-3), 149.2 (C-4), 170.1 (C-1).

Silylation of (Z)-7-O-(tert-butylidiphenylsilyl)-2,3-dideoxy-4,5-O-isopropylidene-D-ribohept-2-enoate (25). A mixture of **25**²² (3 g, 6.28 mmol), AgNO_3 (3.1 g, 18.8 mmol), TBDPSCI (4.9 mL, 18.8 mmol), pyridine (3.0 mL, 37.7 mmol), and CH_3CN (20 mL) was stirred in the dark at rt for 2.5 h. After the reaction mixture was diluted with EtOAc (30 mL), the resulting suspension was filtered through Celite, and the filter cake was washed with EtOAc. The combined filtrate and washings were successively washed with ice-cold 10% aqueous sulfuric acid (20 mL \times 2) and brine (20 mL) and condensed *in vacuo*. The residue was purified by column chromatography (*n*-hexane/EtOAc = 50/1) to give (Z)-6,7-bis-*O*-(tert-butylidiphenylsilyl)-2,3-dideoxy-4,5-*O*-isopropylidene-D-ribohept-2-enoate (**26**, 4.5 g,

6.20 mmol, 99%) as a colourless oil. $[\alpha]_{\text{D}}^{25} -68.4$ ($c = 0.96$, CHCl_3). IR (neat): 1720, 1589, 1473, 1427, 1381, 1222, 1111, 1072 cm^{-1} . $^1\text{H NMR}$ (800 MHz, CDCl_3) δ : 0.96/1.07 [each 9H, s, $(\text{CH}_3)_3\text{CSi}$], 1.35/1.41 [each 3H, s, $(\text{CH}_3)_2\text{C}$], 3.48 (2H, dd, $J = 11.1, 4.0, \text{H-7a}$), 3.61 (3H, s, CO_2CH_3), 3.76 (2H, dd, $J = 11.1, 6.2, \text{H-7b}$), 3.91 (1H, ddd, $J = 7.2, 6.2, 4.0, \text{H-6}$), 4.62 (1H, dd, $J = 7.2, 2.9, \text{H-5}$), 5.54 (1H, dd, $J = 11.5, 1.3, \text{H-2}$), 5.58 (1H, ddd, $J = 8.4, 2.9, 1.3, \text{H-4}$), 5.83 (1H, dd, $J = 11.5, 8.4, \text{H-3}$), 7.24–7.72 (20H, m, arom.). $^{13}\text{C NMR}$ (200 MHz, CDCl_3) δ : 19.1/19.3 [$(\text{CH}_3)_3\text{CSi}$], 24.5/26.5 [$(\text{CH}_3)_2\text{C}$], 26.8(2C)/26.93(2C)/26.96(2C) [$(\text{CH}_3)_3\text{CSi}$], 51.4 (CO_2CH_3), 65.9(C-7), 73.0 (C-4), 73.8 (C-6), 80.0 (C-5), 108.5 [$(\text{CH}_3)_2\text{C}$], 120.7 (C-2), 127.75/127.81/129.85/129.89/135.5/135.6 (each 2C, d, arom.), 132.8/132.3 (each 2C, s, arom.), 145.8 (C-3), 165.6 (C-1). HRMS (ESI) m/z : $[\text{M} + \text{Na}]^+$ calcd for $\text{C}_{43}\text{H}_{54}\text{O}_6\text{NaSi}_2$, 745.3351; found, 745.3347.

Lactonisation of the Bis-O-TBDP ester (26). A mixture of **26** (7.5 g, 10.3 mmol), acetic acid (66 mL), and H_2O (11 mL) was heated at 100 °C for 2 h. After being cooled, the reaction mixture was poured into cold water (300 mL). The resulting mixture was neutralised with sodium hydrogen carbonate and extracted with ethyl acetate (100 mL \times 3). The extract was washed with brine (150 mL) and condensed *in vacuo* to give a pale yellow oil (7.4 g). The residue was purified by column chromatography (*n*-hexane/EtOAc = 100/1) to give (4*S*,5*S*,6*R*)-6,7-bis-*O*-(tert-butylidiphenylsilyl)-5-hydroxy-2-hepten-4-olide (**27**, 3.0 g, 4.56 mmol, 44%) as a colourless oil. $[\alpha]_{\text{D}}^{25} -62.6$ ($c = 0.78$, CHCl_3). IR (neat): 1786, 1747, 1589, 1464, 1427, 1392, 1361, 1080 cm^{-1} . $^1\text{H NMR}$ (800 MHz, CDCl_3) δ : 1.04/1.05 [each 9H, s, $(\text{CH}_3)_3\text{C}$], 2.77 (1H, d, $J = 5.3, \text{OH}$), 3.80 (1H, dd, $J = 10.9, 2.9, \text{H-7a}$), 3.84 (1H, dd, $J = 10.9, 5.1, \text{H-7b}$), 3.84–3.88 (2H, m, H-6 and H-5), 5.13 (1H, ddd, $J = 5.6, 1.9, 1.6, \text{H-4}$), 6.03 (1H, dd, $J = 5.8, 1.9, \text{H-2}$), 7.21 (1H, dd, $J = 5.8, 1.6, \text{H-3}$), 7.27–7.62 (20H, m, arom.). $^{13}\text{C NMR}$ (200 MHz, CDCl_3) δ : 19.1/19.2 [$(\text{CH}_3)_3\text{CSi}$], 26.8/27.0 [each 3C, $(\text{CH}_3)_3\text{CSi}$], 65.0 (C-7), 72.8 (C-5), 73.7 (C-6), 83.1 (C-4), 122.1 (C-2), 127.7/127.8/127.9/128.0/129.9/129.96/130.00/130.2/135.5/135.6/135.7/135.8 (d, arom.), 132.4/132.5/132.6/133.1 (s, arom.), 154.4 (C-3), 172.8 (C-1). HRMS (ESI) m/z : $[\text{M} + \text{Na}]^+$ calcd for $\text{C}_{39}\text{H}_{46}\text{O}_5\text{NaSi}_2$, 673.2276; found, 673.2771.

E1cb elimination reaction of the butenolide (27). A mixture of **27** (3.0 g, 4.60 mmol), Et_3N (1.6 mL, 11.4 mmol), DMAP (567 mg, 4.65 mmol), Bz_2O (0.53 mL, 5.6 mmol), and CH_2Cl_2 (10 mL) was stirred at rt for 0.5 h. The reaction mixture was poured into cold water (30 mL) and extracted with CH_2Cl_2 (10 mL \times 3). The extract was washed with brine (15 mL) and condensed *in vacuo*. The residue was purified by column chromatography (*n*-hexane/EtOAc = 10/1 \rightarrow 5/1) to give (4*Z*,6*S*)-6,7-bis-*O*-(tert-butylidiphenylsilyloxy)-2,4-heptadiene-4-olide (**28**, 728 mg, 1.15 mmol, 25%) and its *E*-isomer **29** (1.66 g, 2.62 mmol, 57%).

Z-Isomer (28): $[\alpha]_{\text{D}}^{25} -26.4$ ($c = 0.72$, CHCl_3). IR (neat): 1789, 1763, 1670, 1469, 1427, 1361, 1056 cm^{-1} . $^1\text{H NMR}$ (800 MHz, CDCl_3) δ : 1.01/1.07 [each 9H, s, $(\text{CH}_3)_3\text{C}$], 3.68 (1H, dd, $J = 10.3, 5.1, \text{H-7a}$), 3.77 (1H, dd, $J = 10.3, 5.1, \text{H-7b}$), 4.98 (1H, dt, $J = 8.9, 5.1, \text{H-6}$), 5.11 (1H, ddd, $J = 8.9, 0.7, 0.3, \text{H-5}$), 6.00 (1H, dd, $J = 5.4, 0.7, \text{H-2}$), 7.02 (1H, dd, $J = 5.4, 0.3, \text{H-3}$), 7.26–7.65 (20H, m, arom.). $^{13}\text{C NMR}$ (200 MHz, CDCl_3) δ : 19.16/19.20



[[CH₃]₃C], 26.7/26.9 [[CH₃]₃C], 67.7 (C-7), 69.2 (C-6), 116.3 (C-5), 120.0 (C-2), 143.3 (C-3), 127.4/127.5/127.6/129.6/129.7/129.8/135.59/135.61/135.8/135.9 (each 2C, d, arom.), 133.17/133.27/133.30/133.7(s, arom.), 148.6 (C-4), 169.2 (C-1). HRMS (ESI) *m/z*: [M + Na]⁺ calcd for C₃₉H₄₄O₄NaSi₂, 655.2670; found, 655.2663.

E-Isomer (29) [α]_D²⁵ -54.5 (*c* = 0.90, CHCl₃). IR (neat): 1782, 1762, 1678, 1470, 1427, 1107 cm⁻¹. ¹H NMR (800 MHz, CDCl₃) δ : 0.88/0.95 [each 9H, s, (CH₃)₃C], 3.62 (1H, dd, *J* = 10.1, 6.6, H-7a), 3.80 (1H, dd, *J* = 10.1, 4.9, H-7b), 4.43 (1H, ddd, *J* = 9.3, 6.6, 4.9, H-6), 5.66 (1H, ddd, *J* = 9.3, 1.8, 0.7, H-5), 5.91 (1H, dd, *J* = 5.6, 1.8, H-2), 6.81 (1H, dd, *J* = 5.6, 0.7, H-3), 7.25–7.61 (20H, m, arom.). ¹³C NMR (200 MHz, CDCl₃) δ : 19.1/19.2 [[CH₃]₃C], 26.7/26.8 [[CH₃]₃C], 67.6 (C-7), 70.1 (C-6), 115.5 (C-5), 120.2 (C-2), 127.7(6C)/129.78/128.88/129.95/135.5/135.6/135.7/135.9 (each 2C, d, arom.), 132.94(2C)/133.1/133.2 (s, arom.), 140.1 (C-3), 150.6 (C-4), 169.4 (C-1). HRMS (ESI) *m/z*: [M + Na]⁺ calcd for C₃₉H₄₄O₄NaSi₂, 655.2670; found, 655.2667.

Desilylation of the Z-isomer (28). A mixture of **28** (63 mg, 99 μ mol), acetic acid (17 μ L, 0.30 mmol), 1 M solution of TBAF in THF (0.25 mL, 0.25 mmol), and THF (1 mL) was stirred at rt for 1 h. The reaction mixture was condensed *in vacuo*, and the residue was purified by column chromatography (*n*-hexane/EtOAc = 1/5) to give (4*Z*,6*S*)-6,7-dihydroxy-2,4-heptadien-4-olide^{30–33} (**S-8**, 15 mg, 96 μ mol, 97%) as a colourless microcrystalline solid. Mp. 65–66 °C, [α]_D²⁴ +12.5 (*c* = 0.5, MeOH). Lit.³⁰ a pale yellow solid, Mp. 87–89 °C, [α]_D²² +53.1 (*c* = 0.42, acetone). Lit.³¹ a colourless liquid, [α]_D³² +12 (*c* = 0.5, MeOH). Lit.³² a colourless viscous liquid, [α]_D²⁹ +8 (*c* = 0.6, MeOH). Lit.³³ colourless oil, [α]_D +13 (*c* = 0.17, CHCl₃). IR (KBr): 1786, 1755, 1674, 1558, 1400, 1303, 1122, 1076, 1030 cm⁻¹. ¹H NMR (800 MHz, CD₃OD) δ : 3.58 (2H, d, *J* = 5.6, H-7a and H-7b), 4.72 (1H, dt, *J* = 8.7, 5.6, H-6), 5.41 (1H, d, *J* = 8.7, H-5), 6.30 (1H, d, *J* = 5.4, H-2), 7.65 (1H, d, *J* = 5.4, H-3). ¹³C NMR (200 MHz, CD₃OD) δ : 66.6 (C-7), 68.6 (C-6), 116.5 (C-5), 121.1 (C-2), 145.9 (C-3), 151.4 (C-4), 171.4 (C-1). HRMS (ESI) *m/z*: [M + Na]⁺ calcd for C₇H₈O₄Na, 179.0315; found, 179.0314.

Desilylation of the E-isomer (29). Following the method similar to that used for the preparation of **S-8**, **29** (230 mg, 0.36 mmol) was desilylated with 1 M solution of TBAF in THF (0.91 mL, 0.91 mmol) in THF (5 mL) in the presence of acetic acid (62 μ L, 1.09 mmol). Work up and column chromatographic purification (*n*-hexane/EtOAc = 1/5) gave (4*E*,6*S*)-6,7-dihydroxy-2,4-heptadien-4-olide³⁴ (**S-9**, 53 mg, 0.94 mmol, 94%) as a colourless oil. [α]_D²⁵ -30.6 (*c* = 0.32, CHCl₃). IR (neat): 1786, 1755, 1670, 1554, 1419, 1300, 1188, 1126, 1072, 1033 cm⁻¹. ¹H NMR (800 MHz, CD₃OD) δ : 3.54 (1H, dd, *J* = 11.2, 5.5, H-7a), 3.60 (1H, dd, *J* = 11.2, 5.8, H-7b), 4.54 (1H, ddd, *J* = 8.3, 5.8, 5.5, H-6), 5.77 (1H, ddd, *J* = 8.3, 1.8, 0.7, H-5), 6.32 (1H, dd, *J* = 5.6, 1.8, H-2), 8.04 (1H, dd, *J* = 5.6, 0.7, H-3). ¹³C NMR (200 MHz, CD₃OD) δ : 66.9 (C-7), 69.4 (C-6), 116.4 (C-5), 121.6 (C-2), 143.0 (C-3), 151.9 (C-4), 171.5 (C-1). HRMS (ESI) *m/z*: [M + Na]⁺ calcd for C₇H₈O₄Na, 179.0315; found, 179.0312.

Acylation of γ -alkylidenebutenolides (1–4). (*S*)- and (*R*)- γ -Alkylidenebutenolides [(*S*)-**1**–(*S*)-**4** and (*R*)-**1**–(*R*)-**4**] were syn-

thesised according to the method reported by us²² and acylated with following reagents.

With acetic anhydride

(4*Z*,6*S*)-6-Acethyloxy-7-benzoyloxy-2,4-heptadien-4-olide [(*S*)-acetyl-melodoriol (**S-10**)]. To a stirred solution of (*S*)-melodoriol (**S-1**, 78 mg, 0.30 mmol) in pyridine (2.0 mL) was added acetic anhydride (57 μ L, 0.60 mmol) at 0 °C, and the resulting mixture was stirred at rt for 4 h. The reaction mixture was poured into cold water (6 mL) and extracted with EtOAc (5 mL \times 3). The extract was successively washed with 10% aqueous H₂SO₄ (6 mL), aqueous NaHCO₃ (6 mL), and brine (6 mL) and condensed *in vacuo*. The residue was purified by column chromatography (*n*-hexane/EtOAc = 10/1) to give the title compound^{30–32,35} [(*S*)-**10**, 79 mg, 0.26 mmol, 87%] as a colourless microcrystalline solid. For analytical purposes, a small portion was purified by recrystallization from a mixture of EtOAc and MeOH to give (**S-10**) as colourless needles. Mp. 75–76 °C, [α]_D²⁷ +54.0 (*c* = 1.0, CHCl₃). Lit.³⁰ colourless crystal, 78–80 °C (from *i*PrOH/*n*-hexane), [α]_D²² +41.0 (*c* = 0.33, CHCl₃). Lit.³¹ [α]_D²² +32.5 (*c* = 2, CHCl₃). Lit.³² a white solid, 78–80 °C (ether/*n*-hexane), [α]_D²⁷ +31.9 (*c* = 0.23, CHCl₃). Lit.³⁵ a colourless liquid, [α]_D²⁵ +42.3 (*c* = 0.7, CHCl₃). IR (KBr): 1782, 1744, 1724, 1678, 1600, 1562, 1373, 1273, 1176, 1026 cm⁻¹. ¹H-NMR (500 MHz, CDCl₃) δ : 2.11 [3H, s, C(O)CH₃], 4.53 (1H, dd, *J* = 11.7, 6.0, H-7a), 4.57 (1H, dd, *J* = 11.7, 4.0, H-7b), 5.34 (1H, d, *J* = 8.0, H-5), 6.15 (1H, ddd, *J* = 8.0, 6.0, 4.0, H-6), 6.29 (1H, d, *J* = 5.4, H-2), 7.38 (1H, d, *J* = 5.4, H-3), 7.44–7.48 (2H, m, arom.), 7.56–7.61 (1H, m, arom.), 8.01–8.05 (2H, m, arom.). ¹³C-NMR (125 MHz, CDCl₃) δ : 20.9 [C(O)CH₃], 64.6 (C-7), 67.2 (C-6), 108.9 (C-5), 121.6 (C-2), 128.4(2C)/129.7(2C)/133.3 (d, arom.), 129.5 (s, arom.), 143.3 (C-3), 150.6 (C-4), 166.0 [C(O)Ph], 168.5 (C-1), 169.8 [C(O)CH₃]. HRMS (ESI) *m/z*: [M + Na]⁺ calcd for C₁₆H₁₄O₆Na, 325.0683; found, 325.0678.

In a similar manner, (*R*)-**1** (105 mg, 0.40 mmol) gave (*R*)-**10** (102 mg, 0.34 mmol, 84%). [α]_D²⁷ -52.9 (*c* = 0.98, CHCl₃), lit.³⁰ [α]_D²⁷ -38.8 (*c* = 1.25, CHCl₃). ¹H and ¹³C-NMR spectral data of (*R*)-**10** agreed well with those of the corresponding antipode [(*S*)-**10**].

(4*Z*,6*S*)-7-Acethyloxy-6-benzoyloxy-2,4-heptadien-4-olide [(*S*)-**13**]. Following the procedure used for the preparation of (*S*)-**10**, isofruticosinol [(*S*)-**3**, 158 mg, 0.61 mmol] was converted to the title compound [(*S*)-**13**, 162 mg, 0.54 mmol, 88%] as a colourless microcrystalline solid. For analytical purposes, a small portion was purified by recrystallization from a mixture of *n*-hexane and EtOAc to give (**S-13**) as colourless needles. Mp. 85–86 °C. [α]_D²⁵ -166.9 (*c* = 0.60, CHCl₃). IR (KBr): 1793, 1778, 1751, 1685, 1566, 1450, 1365, 1285, 1103 cm⁻¹; ¹H-NMR (800 MHz, CDCl₃) δ : 2.08 [3H, s, C(O)CH₃], 4.45 (1H, dd, *J* = 11.8, 4.1, H-7a), 4.48 (1H, dd, *J* = 11.8, 6.4, H-7b), 5.37 (1H, ddd, *J* = 8.1, 0.8, 0.4, H-5), 6.20 (1H, ddd, *J* = 8.1, 6.4, 4.1, H-6), 6.29 (1H, ddd, *J* = 5.5, 0.8, H-2), 7.38 (1H, dd, *J* = 5.5, 0.4, H-3), 7.45–7.48 (2H, m, arom.), 7.57–7.60 (1H, m, arom.), 8.03–8.05 (2H, m). ¹³C-NMR (200 MHz, CDCl₃) δ : 20.7 [C(O)CH₃], 64.1 (C-7), 67.9 (C-6), 108.9 (C-5), 121.6 (C-2), 128.5(2C)/129.8(2C)/133.4 (d, arom.), 129.5 (s, arom.), 143.3 (C-3), 150.7 (C-4), 165.4 [C(O)Ph], 168.5 (C-1), 170.5 [C(O)CH₃]. HRMS (ESI) *m/z*: [M + Na]⁺ calcd for C₁₆H₁₄O₆Na, 325.0683; found, 325.0683.



In a similar manner, (**R**)-**3** (132 mg, 0.51 mmol) gave (**R**)-**13** (142 mg, 0.47 mmol, 93%). $[\alpha]_{\text{D}}^{25} +169.2$ ($c = 0.70$, CHCl_3); ^1H and ^{13}C -NMR spectral data of (**R**)-**13** agreed well with those of the corresponding antipode [(**S**)-**13**].

(4*E*,6*S*)-6-Acethyloxy-7-benzoyloxy-2,4-heptadien-4-olide [(**S**)-**15**]. Following the procedure used for the preparation of **S**-**10**, isomerodorinol [(**S**)-**2**, 480 mg, 1.8 mmol] was converted to the title compound³⁵ [(**S**)-**15**, 427 mg, 1.4 mmol, 76%] as a colourless microcrystalline solid. For analytical purposes, a small portion was purified by recrystallization from a mixture of *n*-hexane and EtOAc to give (**S**)-**15** as colourless needles. Mp. 61–63 °C. $[\alpha]_{\text{D}}^{27} -18.3$ ($c = 0.94$, CHCl_3), lit.³⁵ a white solid, $[\alpha]_{\text{D}}^{27} -11.2$ ($c = 0.5$, CHCl_3). IR (KBr): 1789, 1759, 1724, 1674, 1600, 1369, 1273, 1114, 1029 cm^{-1} . ^1H -NMR (500 MHz, CDCl_3) δ : 2.10 [3H, s, $\text{C}(\text{O})\text{CH}_3$], 4.47 (1H, dd, $J = 11.7$, 6.9, H-7a), 4.53 (1H, dd, $J = 11.7$, 4.3, H-7b), 5.73 (1H, ddd, $J = 10.0$, 2.0, 0.8, H-5), 5.98 (1H, ddd, $J = 10.0$, 6.9, 4.3, H-6), 6.35 (1H, dd, $J = 5.7$, 2.0, H-2), 7.48–7.49 (2H, m, arom.), 7.90 (1H, dd, $J = 5.7$, 0.8, H-3), 7.89–7.92 (1H, m, arom.), 8.00–8.04 (2H, m, arom.). ^{13}C -NMR (125 MHz, CDCl_3) δ : 21.0 [$\text{C}(\text{O})\text{CH}_3$], 65.0 (C-7), 66.7 (C-6), 107.4 (C-5), 122.5 (C-2), 128.6(2C)/129.7(2C)/133.5 (d, arom.), 129.2 (s, arom.), 140.2 (C-3), 153.4 (C-4), 165.9 [$\text{C}(\text{O})\text{Ph}$], 168.7 (C-1), 170.1 [$\text{C}(\text{O})\text{CH}_3$]. HRMS (ESI) m/z : [$\text{M} + \text{Na}$]⁺ calcd for $\text{C}_{16}\text{H}_{14}\text{O}_6\text{Na}$, 325.0683; found, 325.0673.

In a similar manner, (**R**)-**2** (171 mg, 0.66 mmol) gave (**R**)-**15** (158 mg, 0.52 mmol, 79%). $[\alpha]_{\text{D}}^{27} +19.4$ ($c = 1.00$, CHCl_3). ^1H and ^{13}C -NMR spectral data of (**R**)-**15** agreed well with those of the corresponding antipode [(**S**)-**15**].

(4*E*,6*S*)-7-Acetyloxy-6-benzoyloxy-2,4-heptadien-4-olide [(**S**)-**18**]. Following the procedure used for the preparation of (**S**)-**10**, (**S**)-isofrucosinol [(**S**)-**4**, 126 mg, 0.48 mmol] was converted to the title compound [(**S**)-**18**, 127 mg, 0.42 mmol, 87%] as a colourless microcrystalline solid. For analytical purposes, a small portion was purified by recrystallization from a mixture of *n*-hexane and EtOAc to give (**S**)-**18** as colourless needles. Mp. 81–82 °C. $[\alpha]_{\text{D}}^{25} -82.5$ ($c = 1.03$, CHCl_3). IR (KBr): 1789, 1747, 1712, 1636, 1558, 1269, 1176, 1068, 1026 cm^{-1} . ^1H -NMR (800 MHz, CDCl_3) δ : 2.08 [3H, s, $\text{C}(\text{O})\text{CH}_3$], 4.39 (1H, dd, $J = 11.9$, 6.8, H-7a), 4.42 (1H, dd, $J = 11.9$, 4.4, H-7b), 5.74 (1H, ddd, $J = 10.0$, 1.8, 0.7, H-5), 6.07 (1H, ddd, $J = 10.0$, 6.8, 4.4, H-6), 6.36 (1H, dd, $J = 5.6$, 1.8, H-2), 7.45–7.48 (2H, m, arom.), 7.58–7.61 (1H, m, arom.), 7.97 (1H, dd, $J = 5.6$, 0.7, H-3), 8.01–8.03 (2H, m, arom.). ^{13}C -NMR (200 MHz, CDCl_3) δ : 20.7 [$\text{C}(\text{O})\text{CH}_3$], 64.6 (C-7), 67.3 (C-6), 107.3 (C-5), 122.6 (C-2), 128.6 (2C)/129.7(2C)/133.6 (d, arom.), 129.6 (s, arom.), 140.3 (C-3), 153.6 (C-4), 165.6 [$\text{C}(\text{O})\text{Ph}$], 168.6 (C-1), 170.5 [$\text{C}(\text{O})\text{CH}_3$]. HRMS (ESI) m/z : [$\text{M} + \text{Na}$]⁺ calcd for $\text{C}_{16}\text{H}_{14}\text{O}_6\text{Na}$, 325.0683; found, 325.0683.

In a similar manner, (*R*)-isofrucosinol [(**R**)-**4**, 133 mg, 0.51 mmol] gave (**R**)-**16** (147 mg, 0.49 mmol, 90%). $[\alpha]_{\text{D}}^{25} +83.8$ ($c = 1.04$, CHCl_3). ^1H and ^{13}C -NMR spectral data of (**R**)-**16** agreed well with those of the corresponding antipode [(**S**)-**16**].

With pivaloyl chloride

(4*Z*,6*S*)-6-Pivaloyloxy-7-benzoyloxy-2,4-heptadien-4-olide [(**S**)-**11**]. To a stirred solution of (*S*)-melodorinol [(**S**)-**1**, 154 mg, 0.59 mmol] in pyridine (1.0 mL) was added pivaloyl chloride

(145 μL , 1.18 mmol) at 0 °C, and the resulting mixture was stirred at rt for 16 h. The reaction mixture was poured into cold water (20 mL) and extracted with EtOAc (10 mL \times 3). The extract was successively washed with 10% aqueous H_2SO_4 (20 mL), aqueous NaHCO_3 (20 mL), and brine (20 mL) and condensed *in vacuo*. The residue was purified by column chromatography (*n*-hexane/EtOAc = 6/1) to give the title compound [(**S**)-**11**, 108 mg, 0.31 mmol, 53%] as a colourless microcrystalline solid. For analytical purposes, a small portion was purified by recrystallization from a mixture of *n*-hexane and EtOAc to give (**S**)-**11** as colourless needles. Mp. 84–85 °C. $[\alpha]_{\text{D}}^{23} +13.2$ ($c = 1.01$, CHCl_3). IR (KBr): 1774, 1747, 1732, 1674, 1458, 1276, 1149, 1122, 1037 cm^{-1} . ^1H -NMR (800 MHz, CDCl_3) δ : 1.20 [9H, s, $\text{C}(\text{CH}_3)_3$], 4.55 (1H, dd, $J = 11.6$, 4.3, H-7a), 4.57 (1H, dd, $J = 11.6$, 6.5, H-7b), 5.30 (1H, br d like, $J = ca.$ 7.9, H-5), 6.11 (1H, ddd, $J = 7.9$, 6.5, 4.3, H-6), 6.28 (1H, dd like, $J = 5.5$, 0.5, H-2), 7.38 (1H, d, $J = 5.5$, H-3) 7.43–7.46 (2H, m, arom.), 7.56–7.68 (1H, m, arom.), 8.01–8.02 (2H, m, arom.). ^{13}C -NMR (200 MHz, CDCl_3) δ : 27.0 [$\text{C}(\text{CH}_3)_3$], 38.8 [$\text{C}(\text{CH}_3)_3$], 64.4 (C-7), 67.2 (C-6), 109.1 (C-5), 121.5 (C-2), 128.5(2C)/129.8 (2C)/133.5 (d, arom.), 129.5 (s, arom.), 143.3 (C-3), 150.6 (C-4), 165.6 [$\text{C}(\text{O})\text{Ph}$], 168.5 (C-1), 177.3 [$\text{C}(\text{O})\text{C}(\text{CH}_3)_3$]. HRMS (ESI) m/z : [$\text{M} + \text{Na}$]⁺ calcd for $\text{C}_{19}\text{H}_{20}\text{O}_6\text{Na}$, 367.1152; found, 367.1150.

In a similar manner, (**R**)-**1** (76 mg, 0.29 mmol) gave (**R**)-**11** (58 mg, 0.17 mmol, 58%). $[\alpha]_{\text{D}}^{27} -11.6$ ($c = 0.95$, CHCl_3). ^1H and ^{13}C -NMR spectral data of (**R**)-**11** agreed well with those of the corresponding antipode [(**S**)-**11**].

(4*Z*,6*S*)-6-Benzoyloxy-7-pivaloyloxy-2,4-heptadien-4-olide [(**S**)-**14**]. Following the procedure used for the preparation of (*S*)-pivaloylmerodorinol [(**S**)-**11**], (*S*)-frucosinol [(**S**)-**3**, 103 mg, 0.40 mmol] was converted to the title compound [(**S**)-**14**, 124 mg, 0.36 mmol, 91%] as a colourless oil. $[\alpha]_{\text{D}}^{25} -149.0$ ($c = 0.92$, CHCl_3). IR (neat): 1786, 1751, 1728, 1681, 1454, 1315, 1265, 1107, 1026 cm^{-1} . ^1H -NMR (800 MHz, CDCl_3) δ : 1.17 [9H, s, $\text{C}(\text{CH}_3)_3$], 4.42 (1H, dd, $J = 11.7$, 4.2, H-7a), 4.48 (1H, dd, $J = 11.7$, 6.8, H-7b), 5.35 (1H, br d like, $J = ca.$ 8.1, H-5), 6.24 (1H, ddd, $J = 8.1$, 6.8, 4.2, H-6), 6.30 (1H, d, $J = 5.5$, 0.6, H-2), 7.38 (1H, d, $J = 5.5$, H-3), 7.43–7.47 (2H, m, arom.), 7.56–7.60 (1H, m, arom.), 8.02–8.05 (2H, m, arom.). ^{13}C -NMR (200 MHz, CDCl_3) δ : 27.1 [$\text{C}(\text{CH}_3)_3$], 38.9 [$\text{C}(\text{CH}_3)_3$], 63.8 (C-7), 68.0 (C-6), 109.0 (C-5), 121.7 (C-2), 128.5(2C)/129.7(2C)/133.3 (d, arom.), 129.5 (s, arom.), 143.2 (C-3), 150.8 (C-4), 165.3 [$\text{C}(\text{O})\text{Ph}$], 168.5 (C-1), 177.9 [$\text{C}(\text{O})\text{C}(\text{CH}_3)_3$]. HRMS (ESI) m/z : [$\text{M} + \text{Na}$]⁺ calcd for $\text{C}_{19}\text{H}_{20}\text{O}_6\text{Na}$, 367.1152; found, 367.1144.

In a similar manner, (**R**)-**3** (104 mg, 0.40 mmol) gave (**R**)-**14** (119 mg, 0.35 mmol, 87%). $[\alpha]_{\text{D}}^{25} +148.6$ ($c = 0.88$, CHCl_3). ^1H and ^{13}C -NMR spectral data of (**R**)-**14** agreed well with those of the corresponding antipode [(**S**)-**14**].

(4*E*,6*S*)-7-Benzoyloxy-6-pivaloyloxy-2,4-heptadien-4-olide [(**S**)-**16**]. Following the procedure used for the preparation of (*S*)-pivaloylmerodorinol [(**S**)-**11**], (*S*)-isomelodorinol [(**S**)-**2**, 91 mg, 0.35 mmol] was converted to the title compound [(**S**)-**16**, 102 mg, 0.30 mmol, 85%] as a colourless microcrystalline solid. For analytical purposes, a small portion was purified by recrystallization from a mixture of *n*-hexane and EtOAc give the



(S)-16 as colourless needles. Mp. 94–95 °C. $[\alpha]_{\text{D}}^{22} -35.6$ ($c = 0.70$, CHCl_3). IR (KBr): 1790, 1751, 1720, 1678, 1604, 1454, 1396, 1153, 1126, 1037 cm^{-1} . $^1\text{H-NMR}$ (800 MHz, CDCl_3) δ : 1.18 [9H, s, $(\text{CH}_3)_3\text{CO}$], 4.51 (1H, dd, $J = 11.7$, 6.9, H-7a), 4.53 (1H, dd, $J = 11.7$, 4.6, H-7b), 5.71 (1H, ddd, $J = 10.1$, 1.8, 0.8, H-5), 5.94 (1H, ddd, $J = 10.1$, 6.9, 4.6, H-6), 6.35 (1H, dd, $J = 5.7$, 1.8, H-2), 7.46 (2H, m, arom), 7.59 (1H, m, arom), 7.92 (1H, dd, $J = 5.7$, 0.8, H-3), 8.00 (2H, m, arom). $^{13}\text{C-NMR}$ (200 MHz, CDCl_3) δ : 64.8 (C-7), 66.5 (C-6), 107.3 (C-5), 122.4 (C-2), 128.4/129.5/133.3 (d, arom.), 129.2 (s, arom.), 140.1 (C-3), 153.4 (C-4), 165.8 $[\text{C}(\text{O})\text{Ph}]$, 168.6 (C-1), 177.5 [9H, s, $(\text{CH}_3)_3\text{CO}$]. HRMS (ESI) m/z : $[\text{M} + \text{Na}]^+$ calcd for $\text{C}_{19}\text{H}_{20}\text{O}_6\text{Na}$, 367.1149; found, 367.1152.

In a similar manner, **(R)-2**, (121 mg, 0.46 mmol) gave **(R)-16** (132 mg, 0.39 mmol, 83%). $[\alpha]_{\text{D}}^{22} 35.4$ ($c = 0.91$, CHCl_3); ^1H and $^{13}\text{C-NMR}$ spectral data of **(R)-16** agreed well with those of the corresponding antipode **[(S)-16]**.

(4E,6S)-6-Benzoyloxy-7-pivaloyloxy-2,4-heptadien-4-olide **[(S)-19]**. Following the procedure used for the preparation of (S)-pivaloylmerodorinol **[(S)-11]**, (S)-isofruticosinol **[(S)-4]**, 103 mg, 0.40 mmol was converted to the title compound **[(S)-19]**, 117 mg, 0.34 mmol, 85% as a colourless oil. $[\alpha]_{\text{D}}^{25} -59.8$ ($c = 0.94$, CHCl_3). IR (neat): 1789, 1752, 1728, 1674, 1600, 1562, 1396, 1265, 1111, 1026 cm^{-1} . $^1\text{H-NMR}$ (800 MHz, CDCl_3) δ : 1.17 [9H, s, $\text{C}(\text{CH}_3)_3$], 4.39 (1H, dd, $J = 5.2$, 4.8, H-7a), 4.41 (1H, dd, $J = 6.1$, 4.8, H-7b), 5.75 (1H, ddd like, $J = ca. 10.0$, 1.8, 0.7, H-5), 6.09 (1H, ddd, $J = 10.0$, 6.1, 5.2, H-6), 6.36 (1H, dd, $J = 5.6$, 1.8, H-2), 7.44–7.48 (2H, m, arom.), 7.57–7.61 (1H, m, arom.), 7.98 (1H, br dd like, $J = ca. 5.6$, 0.7, H-3), 7.99–8.02 (2H, m, arom.). $^{13}\text{C-NMR}$ (200 MHz, CDCl_3) δ : 27.1 $[\text{C}(\text{CH}_3)_3]$, 38.9 $[\text{C}(\text{CH}_3)_3]$, 64.4 (C-7), 67.3 (C-6), 107.5 (C-5), 122.6 (C-2), 128.6 (2C)/129.7(2C)/133.6 (d, arom.), 129.3 (s, arom.), 140.3 (C-3), 153.6 (C-4), 165.6 $[\text{C}(\text{O})\text{Ph}]$, 168.7 (C-1), 178.0 $[\text{C}(\text{O})\text{C}(\text{CH}_3)_3]$. HRMS (ESI) m/z : $[\text{M} + \text{Na}]^+$ calcd for $\text{C}_{19}\text{H}_{20}\text{O}_6\text{Na}$, 367.1152; found, 367.1147.

In a similar manner, **(R)-4** (97 mg, 0.37 mmol) gave **(R)-19** (102 mg, 0.30 mmol, 79%). $[\alpha]_{\text{D}}^{25} +60.9$ ($c = 1.01$, CHCl_3); ^1H and $^{13}\text{C-NMR}$ spectral data of **(R)-19** agreed well with those of the corresponding antipode **[(S)-19]**.

With benzoyl chloride

(4Z,6S)-6,7-Bis(benzoyloxy)-2,4-heptadiene-4-olide **[(S)-12]**. To a stirred solution of **(S)-1** (97 mg, 0.37 mmol) in pyridine (2.3 mL) was added benzoyl chloride (65 μL , 0.56 mmol) at 0 °C, and the resulting mixture was stirred at rt for 0.5 h. The reaction mixture was poured into cold water (10 mL) and extracted with EtOAc (10 mL \times 3). The extract was successively washed with 10% aqueous H_2SO_4 (10 mL), aqueous NaHCO_3 (10 mL), and brine (10 mL) and condensed *in vacuo*. The residue was purified by column chromatography (*n*-hexane/EtOAc = 3/1) to give the title compound^{32,34} **[(S)-12]**, 104 mg, 0.28 mmol, 89% as a colourless microcrystalline solid. For analytical purposes, a small portion was purified by recrystallization from a mixture of *n*-hexane and EtOAc to give **(S)-12** as colourless needles. Mp. 60–62 °C. $[\alpha]_{\text{D}}^{21} -83.4$ ($c = 1.00$, CHCl_3). Lit.³² $[\alpha]_{\text{D}}^{27} -60.8$ ($c = 1.00$, CHCl_3). Lit.³⁴ a colourless oil, $[\alpha]_{\text{D}}^{27} -52.3$ ($c = 0.30$, CHCl_3). IR (KBr): 1782, 1750, 1717, 1685, 1559,

1316, 1267, 1177, 1098, 1087 cm^{-1} . $^1\text{H-NMR}$ (800 MHz, CDCl_3) δ : 4.69 (1H, dd, $J = 11.7$, 4.2, H-7a), 4.72 (1H, dd, $J = 11.7$, 6.2, H-7b), 5.45 (1H, br d like, $J = ca. 8.2$, H-5), 6.29 (1H, dd, $J = 5.4$, 0.5, H-2), 6.37 (1H, ddd, $J = 8.2$, 6.2, 4.2, H-6), 7.40 (1H, d, $J = 5.4$, H-3), 7.42–7.45 (4H, m, arom.), 7.55–7.58 (2H, m, arom.), 8.02–8.06 (4H, m, arom.). $^{13}\text{C-NMR}$ (200 MHz, CDCl_3) δ : 64.6 (C-7), 68.0 (C-6), 108.9 (C-5), 121.6 (C-2), 128.5(4C)/129.7(2C)/129.8(2C)/133.3/133.4 (d, arom.), 129.4/129.5 (s, arom.), 143.3 (C-3), 150.8 (C-4), 165.4/166.0 $[\text{C}(\text{O})\text{Ph}]$, 168.5 (C-1). HRMS (ESI) m/z : $[\text{M} + \text{Na}]^+$ calcd for $\text{C}_{21}\text{H}_{16}\text{O}_6\text{Na}$ 387.0839; found 387.0832.

In a similar manner, **(R)-1** (102 mg, 0.39 mmol) gave **(R)-12** (101 mg, 0.28 mmol, 71%). $[\alpha]_{\text{D}}^{21} +89.7$ ($c = 1.00$, CHCl_3). ^1H and $^{13}\text{C-NMR}$ spectral data of **(R)-12** agreed well with those of the corresponding antipode **[(S)-12]**.

(4E,6S)-7,6-Bis(benzoyloxy)-2,4-heptadiene-4-olide **[(S)-17]**. Following the procedure used for the preparation of **(S)-12**, isomelodorinol **[(S)-2]**, 101 mg, 0.39 mmol was converted to the title compound³³ **[(S)-17]**, 133 mg, 0.37 mmol, 94% as colourless needles. For analytical purposes, a small portion was purified by recrystallization from a mixture of *n*-hexane and EtOAc to give **(S)-17** as colourless needles. Mp. 69–70 °C, $[\alpha]_{\text{D}}^{21} -76.3$ ($c = 1.00$, CHCl_3), lit.³⁴ a pale yellow oil, $[\alpha]_{\text{D}}^{27} -24.9$ ($c = 0.30$, CHCl_3). IR (KBr): 1790, 1751, 1678, 1564, 1317, 1277, 1179, 1030 cm^{-1} . $^1\text{H-NMR}$ (800 MHz, CDCl_3) δ : 4.64 (1H, dd, $J = 11.9$, 6.6, H-7a), 4.67 (1H, dd, $J = 11.9$, 4.4, H-7b), 5.84 (1H, ddd, $J = 10.8$, 1.8, 0.7, H-5), 6.21 (1H, ddd, $J = 10.8$, 6.6, 4.4, H-6), 6.36 (1H, dd, $J = 5.7$, 1.8, H-2), 7.42–7.46 (4H, m, arom.), 7.56–7.59 (2H, m, arom.), 8.02 (1H, dd, $J = 5.7$, 0.7, H-3), 8.01–8.03 (4H, m, arom.). $^{13}\text{C-NMR}$ (200 MHz, CDCl_3) δ : 65.1 (C-7), 67.4 (C-6), 107.4 (C-5), 122.6 (C-2), 128.5(2C)/128.5(2C)/129.7(2C)/129.7(2C)/133.4/133.6 (d, arom.), 129.2/129.3 (s, arom.), 140.3 (C-3), 153.7 (C-4), 165.6/166.0 $[\text{C}(\text{O})\text{Ph}]$, 168.6 (C-1). HRMS (ESI) m/z : $[\text{M} + \text{Na}]^+$ calcd for $\text{C}_{21}\text{H}_{16}\text{O}_6\text{Na}$ 387.0839; Found 387.0839.

In a similar manner, **(R)-2** (100 mg, 0.38 mmol) gave **(R)-17** (136 mg, 0.37 mmol, 98%). $[\alpha]_{\text{D}}^{25} +76.8$ ($c = 1.00$, CHCl_3). ^1H and $^{13}\text{C-NMR}$ spectral data of **(R)-17** agreed well with those of the corresponding antipode **[(S)-17]**.

Dess–Martin oxidation of (S)-melodorinol (S-1) and (S)-isomelodorinol (S-2)

(4Z)-7-Benzoyloxy-6-oxo-2,4-heptadien-4-olide [melodorinone **(20)**]. A mixture of **(S)-1** (100 mg, 0.38 mmol), Dess–Martin periodinane (326 mg, 0.77 mmol), NaHCO_3 (81 mg, 0.96 mmol), and CH_2Cl_2 (5.0 mL) was stirred at rt for 2.5 h. The reaction mixture was quenched with aqueous NaHCO_3 (5 mL), and extracted with CH_2Cl_2 (5 mL \times 3). The extract was washed with brine (10 mL) and condensed *in vacuo*. The residue was purified by column chromatography (*n*-hexane/EtOAc = 2/1) to give the title compound (81 mg, 0.31 mmol, 82%) as a colourless microcrystalline solid. For analytical purposes, a small portion was purified by recrystallization from a mixture of *n*-hexane and EtOAc give the title compound **(20)** as a microcrystalline solid. Mp. 134–136 °C, lit.²⁸ colourless bulky crystals, Mp. 136–138 °C. IR (KBr): 1788, 1730, 1705, 1649, 1491, 1379, 1178, 1014 cm^{-1} . $^1\text{H-NMR}$ (600 MHz, CDCl_3) δ : 5.40 (2H, s, H-7ab), 5.71 (1H, s, H-5), 6.52 (1H, d, $J = 5.6$, H-2), 7.47 (2H, t, J



= 7.2, arom.), 7.55 (1H, d, $J = 5.6$, H-3), 7.59 (1H, t, $J = 7.2$, arom.), 8.11 (2H, d, $J = 7.2$, arom.). $^{13}\text{C-NMR}$ (150 MHz, CD_3Cl_3) δ : 69.3 (C-7), 107.3 (C-5), 124.2 (C-2), 128.4/129.9/133.3 (d, arom.), 129.3 (s, arom.), 145.0 (C-3), 155.9 (C-4), 165.8 [C(O)Ph], 166.9 (C-1), 190.8 (C-6). HRMS (ESI) m/z : $[\text{M} + \text{K}]^+$ calcd for $\text{C}_{14}\text{H}_{10}\text{O}_5\text{K}$ 297.0160; found 297.0151.

(4*E*)-7-Benzoyloxy-6-oxo-2,4-heptadien-4-olide [*melodorinone B* (21)]. Following the procedure used for the preparation of 20, (S)-2 (176 mg, 0.68 mmol) was converted to the title compound (21, 150 mg, 0.58 mmol, 86%) as a colourless microcrystalline solid. For analytical purposes, a small portion was purified by recrystallization from a mixture of *n*-hexane and EtOAc to give 21 as colourless needles. Mp. 143–145 °C. Lit.²⁸ colourless bulky crystals, Mp. 139–140 °C. IR (KBr): 1784, 1730, 1703, 1678, 1628, 1410, 1313, 1111, 1095, 1014, cm^{-1} . $^1\text{H-NMR}$ (600 MHz, CDCl_3) δ : 5.01 (2H, s, H-7ab), 6.35 (1H, br s like, H-5), 6.52 (1H, d, $J = 5.5$, H-2), 7.49 (2H, t, $J = 7.2$, arom.), 7.62 (1H, t, $J = 7.2$, arom.), 8.11 (2H, d, $J = 7.2$, arom.), 8.33 (1H, d, $J = 5.5$, H-3). $^{13}\text{C-NMR}$ (150 MHz, CDCl_3) δ : 69.2 (C-7), 103.1 (C-5), 125.9 (C-2), 128.9(2C)/129.9(2C)/133.7 (d, arom.), 128.8 (s, arom.), 142.7 (C-3), 160.5 (C-4), 165.8 [C(O)Ph], 167.4 (C-1), 190.0 (C-6). HRMS (ESI) m/z : $[\text{M} + \text{Na}]^+$ calcd for $\text{C}_{14}\text{H}_{10}\text{O}_5\text{Na}$ 281.0420; found 281.0414.

Biology

Reagents for bioassay. Dulbecco's modified Eagle's medium (DMEM, 4.5 g L^{-1} glucose) was purchased from Sigma-Aldrich (St Louis, MO, USA); fetal bovine serum (FBS), penicillin, and streptomycin were purchased from Gibco (Invitrogen, Carlsbad, CA, USA); and the other chemicals were purchased from Wako Pure Chemical Co., Ltd (Osaka, Japan). The 48-well multiplate and 96-well microplate (Sumilon) were purchased from Sumitomo Bakelite Co., Ltd (Tokyo, Japan).

Cell culture. Murine B16 melanoma 4A5 cells (RCB0557)³⁶ were obtained from Riken Cell Bank (Tsukuba, Japan), and the cells were grown in DMEM supplemented with 10% FBS, penicillin G (100 units per mL), and streptomycin (100 $\mu\text{g mL}^{-1}$) at 37 °C in 5% CO_2 /air. The cells were harvested by incubation in phosphate-buffered saline (–) [PBS (–)] containing 0.05% (w/v) EDTA and 0.02% trypsin for approximately 3 min at 37 °C and were used for subsequent bioassays.

Melanogenesis. The effects of compounds on melanogenesis were examined using B16 melanoma 4A5 cells according to the previously described protocol.³⁷ The melanoma cells ($8.0 \mu \times 10^3$ cells per 200 μL per well) were seeded into 48-well multiplates. After 24 h of culture, a test compound and 1 mM theophylline were added and incubated for 72 h. After incubation, the medium was removed, and 105 μL per well of distilled water was added. Then the cells were homogenised by sonication and lysed with 6 M NaOH (20 μL per well).

An aliquot (100 μL) of the lysate was transferred to a 96-well microplate, and the optical density of each well was measured with a microplate reader (SH-9000, CORONA) at 405 nm (reference: 655 nm). The test compound was dissolved in DMSO, and the final concentration in the medium was 0.1%. The

rates of melanin production were corrected based on the viability of melanoma cells.

Inhibition (%) was calculated using the following formula, where *A* and *B* indicate the optical density of vehicle-treated and test compound-treated groups, respectively, and *C* indicates cell viability (%) (*vide infra*).

$$\text{Inhibition (\%)} = [(A - B)/A]/(C/100) \times 100$$

IC_{50} values were determined graphically on figures including only non-toxic concentrations of compounds.

Viability of melanoma cells. Cell viability was assessed according to the protocol in our previous report³⁷ with slight modification. The melanoma cells (4.0×10^3 cells per 100 μL per well) were seeded into 96-well microplates and incubated for 24 h. After 72 h of incubation with 1 mM theophylline and a test compound, 10 μL of 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT) {5 mg mL^{-1} in [PBS (–)]} solution was added to the medium. After 4 h of incubation, the medium was removed, and 100 μL of isopropanol containing 0.04 M HCl was added to dissolve formazan produced in the cells. The optical density of the formazan was measured at a wavelength of 570 nm (reference: 655 nm). The test compound was dissolved in DMSO, and the final concentration in the medium was 0.1%.

Cell viability (%) was calculated using the following formula, where *A* and *B* indicate the optical density of vehicle-treated and test compound-treated groups, respectively.

$$\text{Cell viability (\%)} = B/A \times 100$$

Author contributions

Conceptualization, GT and TM; Synthesis and structural analysis, KT, RS, NM, YF, RF, and SM; biological experiments and evaluation, FI, YM; writing – original draft preparation, KT and FI; review and editing, GT and TM. The manuscript was reviewed and approved by all authors.

Data availability

All data associated with this study have been included in either the manuscript or in the ESI† associated with the manuscript.

Conflicts of interest

The authors declare that they have no known competing financial interest or personal relationships that could have appeared to influence the work reported in this paper.



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