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Dactyloditerpenol acetate, a new prenylbisabolane-type diterpene from *Aplysia dactylomela* with significant *in vitro* anti-neuroinflammatory activity

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Abstract

A new regular diterpene possessing an unusual 1,6-*anti*-3-methylcyclohex-2-en-1-ol ring system, dactyloditerpenol acetate (**1**), has been extracted from the tropical sea hare *Aplysia dactylomela* and its stereostructure elucidated by spectroscopic methods. The absolute configuration of **1** was determined as 1*S*, 6*S*, 7*R*, 10*S*, and 11*R* by application of Kishi's method for the assignment of absolute configuration of alcohols. The new diterpene potently inhibited *in vitro* thromboxane B₂ (TXB₂) (IC₅₀ 0.4 μM) and superoxide anion (O₂^{•−}) (IC₅₀ 1 μM) generation from *E. coli* lipopolysaccharide (LPS)-activated rat neonatal microglia, with concomitant low short-term toxicity.

Keywords

Aplysia dactylomela; diterpene; prenylbisabolane; anti-neuroinflammatory activity; tuberculosis

Marine organisms, comprising more than half of the total global diversity, offer an enormous and biodiverse source of novel and pharmacologically active compounds.¹ Mollusks belonging to the order Anaspeidea have provided a wealth of structurally diverse natural products with potential application as therapeutic agents.² The sea hare *Aplysia dactylomela* is found worldwide in tropical to warm temperature waters. Previous chemical studies on this species from our group had led to the discovery of aplysqualenols A and B, two squalene-derived polyethers with interesting biological activity.³

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Supplementary data

Underwater photograph of *Aplysia dactylomela*, full spectral data in CDCl₃ for dactyloditerpenol acetate (**1**), ¹H and ¹³C NMR spectra for **3**, Tables 3 and 4, and Fig. 5. Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/>

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As part of our ongoing search for bioactive natural products, we herein report the isolation and structural elucidation of a new prenylbisabolane-type diterpene named dactyloditerpenol acetate (**1**), along with seven known metabolites. Prenylbisabolane-based diterpenoids are very rare in Nature, and to the best of our knowledge, only nine compounds of this type have been thus far described from both marine and terrestrial sources.⁴ Arguably, the most prominent example of this intriguing family of metabolites is laurenditerpenol (**2**), an irregular diterpene from the red alga *Laurencia intricata*, which was found to inhibit HIF-1 by blocking the induction of the oxygen-regulated HIF-1 α protein.⁵ The absolute stereostructure of diterpene **2**, which inhibited HIF-1 activation in T47D breast tumor cells by hypoxia with IC₅₀ value of 0.4 μ M, was settled by a highly convergent asymmetric total synthesis that also clarified the stereochemistry of its conspicuous 1,6-*syn*-3-methylcyclohex-2-en-1-ol ring system.^{6,7}

Spurred by a keen interest for the discovery of new secondary metabolites with biological activity of relevance, compound **1** was evaluated for *in vitro* cytotoxicity against A2058 (melanoma) and DU-145 (prostate) cancer cell lines, antibacterial activity against *Mycobacterium tuberculosis* (H37Rv), and anti-neuroinflammatory activity in *Escherichia coli* lipopolysaccharide (LPS)-activated rat brain microglia, specifically for thromboxane B₂ (TXB₂) and superoxide anion (O₂⁻) generation inhibition. Enhanced generation of O₂⁻ and TXB₂ by LPS-activated microglia, a nervous system mononuclear phagocyte involved in neuroinflammation, has been reported both *in vitro*⁸ and *in vivo*.⁹ Thus, modulation of O₂⁻ and TXB₂ release by LPS-activated microglia has been hypothesized as a therapeutic approach to ameliorate neuroinflammatory disorders.¹⁰

About forty small specimens of the tropical sea hare *Aplysia dactylomela* (Rang, 1828), collected by hand at a depth of 1–2 ft off Mona Island, Puerto Rico, were freeze-dried, homogenized with a 1:1 mixture of MeOH–CHCl₃, and filtered *in vacuo*.¹¹ Preliminary *in vitro* evaluation of cytotoxicity of the *n*-hexane, CHCl₃, and EtOAc extracts was not possible due to a spurious contamination during the assays.¹² Notwithstanding, a portion of the *n*-hexane extract was subjected successively to bioassay-guided fractionation using normal-phase silica gel column chromatography with a mixture of *n*-hexane–EtOAc as eluent to afford pure dactyloditerpenol acetate (**1**, 19 mg).¹³ An array of known compounds co-isolated during this investigation, namely, brominated indol,¹⁴ allolaurinterol,¹⁵ elatol,¹⁶ *trans*-laurediol,¹⁷ parguerol,¹⁸ parguerol 16-acetate,¹⁸ and deoxyparguerol,¹⁸ was confidently identified from comparisons of their spectroscopic data with those previously described in the literature.

Dactyloditerpenol acetate (**1**) was obtained as a colorless oil whose molecular formula was established as C₂₂H₃₈O₄ by HRESI-MS (*m/z* 389.2664 [M+Na]⁺, calcd 389.2668), indicating four degrees of unsaturation. The presence of hydroxyl groups was implied from the broad stretch at 3418 cm⁻¹ in the IR spectrum. The ¹³C NMR and DEPT data (Table 1)

showed resonances for four quaternary, six methine, six methylene, and six methyl carbons. Inspection of the ^1H – ^1H COSY, HSQC and HMBC spectra indicated the presence of an acetate group [δ_{C} 171.2, C-21; 21.1, C-22; δ_{H} 2.10, (3H, s, H₃-22)], two trisubstituted double bonds [δ_{C} 137.3, C-3; 125.6, C-2; δ_{H} 5.37 (1H, br s, H-2); δ_{C} 132.1, C-15; 124.1, C-14; δ_{H} 5.09 (1H, br t, J = 7.1 Hz, H-14)], two oxymethines [δ_{C} 69.0, C-1; δ_{H} 4.00 (1H, br s, H-1) and δ_{C} 79.2, C-10; δ_{H} 4.85 (1H, dd, J = 9.7, 3.5 Hz, H-10)], a secondary methyl at δ_{H} 0.79 (3H, d, J = 6.9 Hz, H₃-19), and four singlet-methyl protons (δ_{H} 1.68, 1.66, 1.61, and 1.15). Two isolated ^1H – ^1H spin systems were quickly established from interpretation of the ^1H – ^1H COSY spectrum (Fig. 1). These segments were in turn interconnected through the long-range ^1H – ^{13}C couplings detected in the HMBC spectrum and summarized in Table 1.

Further analysis of the 2D-NMR data revealed that the two terminal vinyl methyls at δ_{H} 1.61 and 1.68 (H₃-16 and H₃-17) showed HMBC correlations to δ_{C} 124.1 (C-14) and 132.1 (C-15). The sp^2 methine at δ_{H} 5.09 (H-14) showed a COSY correlation to the aliphatic methylene protons at δ_{H} 2.12–2.02 (H₂-13), which in turn showed COSY correlations to a pair of diastereotopic protons at δ_{H} 1.43/1.54 (H₂-12). These NMR data established a terminal isoprene unit, which was expanded to two adjacent asymmetric centers by the observation of HMBC correlations from the methyl singlet at δ_{H} 1.15 (H₃-18) to δ_{C} 79.2 (C-10), 74.1 (C-11) and 37.6 (C-12), and from cross peaks between the oxyacetyl methine proton at δ_{H} 4.85 (H-10) and δ_{C} 31.7 (C-8), 27.1 (C-9), 74.1 (C-11), 37.6 (C-12), 23.5 (C-18), and 171.2 (C-21). These correlations also established unambiguously the locus of the acetate group at C-10. A series of COSY correlations between H-10 and two contiguous aliphatic methylenes at δ_{H} 1.64–1.60 (2H, br m, H₂-9) and 1.26–1.24 (2H, br m, H₂-8) enabled us to further elongate the aliphatic chain. Key HMBC correlations from H₃-19 to C-6 and C-8 helped us place the secondary methyl at C-7. Moreover, HMBC correlations from the oxymethine proton at δ_{H} 4.00 (1H, br s, H-1) to δ_{C} 137.3 (C-3), 125.6 (C-2), and 31.1 (C-7); from the sp^2 methine proton at δ_{H} 5.37 (1H, br s, H-2) to δ_{C} 46.8 (C-6) and 23.1 (C-20), and from the methyl singlet at δ_{H} 1.66 (3H, s, H₃-20) to δ_{C} 125.6 (C-2) and 30.3 (C-4), revealed the presence in **1** of a 3-methylcyclohex-2-en-1-ol ring moiety similar to that present in laurenditerpenol (**2**). On the basis of the analyses outlined above, the planar structure of dactyloditerpenol acetate was elucidated as depicted in **1**.

Five out of the 22 carbons of dactyloditerpenol acetate are stereogenic, and the location of three of them along a C₁₀ acyclic tail made the configurational assignment somewhat difficult. The 2D NOESY experiment allowed us to reduce the number of possible stereoisomers about the 3-methylcyclohex-2-en-1-ol and monoacetylated 1,2-glycol systems within the structure of **1** (Fig. 2). Indeed, the NOESY cross-peaks between the allylic hydroxyl proton at δ_{H} 4.00 (H-1) and the signals at δ_{H} 1.89 (H-7) and 0.79 (H₃-19), combined with the fact that no cross-peaks were detected between H-1 and H-6 (δ_{H} 1.26), clearly pointed to a *trans*-diaxial orientation of the H-1 and H-6 protons. The coupling constant for H-1 and H-6 (J = 8.7 Hz) measured in Bz- d_6 (see Table 3 in Supplementary data) is in full agreement with their *trans*-diaxial orientation. Based on this characterization and comparison of the C-1/H-1 and C-2/H-2 NMR chemical shift values of natural product **1** with reported spectroscopic data of similar systems,¹⁹ it became clear that dactyloditerpenol

acetate must possess a 1,6-*anti*-3-methylcyclohex-2-en-1-ol functionality within its structure. Interestingly, while the cross-peaks of H-6 with H-7 and H₃-19 observed in the NOESY spectrum could not be used to unambiguously assign the relative configuration at C-7 due in part to signal overlap near the 1.26 ppm region of the ¹H NMR spectrum, the spatial coupling of H-7 with H-10 indicated the 7*R** relative arrangement (*vide infra*).²⁰ Analogously, the *anti* relationship between the tertiary hydroxy and acetate groups in **1** was deduced from the additional spatial couplings of H-10 at δ_{H} 4.85 and the signals ascribed to H₃-16 (δ_{H} 1.61), H₃-18 (δ_{H} 1.15), and H₂-12 (δ_{H} 1.54 and 1.43) (Fig. 2). Moreover, deacetylation of **1** with KCN in MeOH at 25 °C afforded glycol **3** in 26% isolated yield (Scheme 1),^{21,22} and when comparisons were made of the ¹³C NMR spectroscopic data of natural product **1** and derivative **3** with reported spectroscopic data of similar systems, we concluded that a 1,2-*anti*-glycol terpenoid structural unit [δ_{C} 79.2 (C-10) and 74.1 (C-11)] in **1** and 78.9 (C-10) and 74.8 (C-11) in **3**, would exhibit the characteristic ¹³C NMR signal difference in chemical shift values.²³ Additionally, by comparison of the carbon shifts of C-12 (δ_{C} 37.6) and C-18 (δ_{C} 23.5) of **1** with those of environmentally similar carbons of previously reported squalene-derived metabolites,^{23d} it was suggested that **1** should possess a 10,11-*erythro* relative configuration. On the basis of the above observations, the relative configuration at C-10 and C-11 in **1** was assigned as *S** and *R**, respectively.

At this stage, reduction of the number of stereoisomers was accomplished by application of the modified Kishi method to assess the absolute configuration at the two stereogenic centers bearing hydroxyl groups in **1**, namely, C-1 and C-11.²⁴ Upon analysis of the ¹³C NMR chemical shifts of the carbons adjacent to the secondary and tertiary alcoholic centers in the presence of chiral lanthanide shift reagents, the absolute configuration of C-1 and C-11 were established as *S* and *R*, respectively (Table 2 and Fig. 3). These data, coupled with our proposed assignments of relative configuration on the basis of proton-proton coupling constant and NOESY data (Fig. 2), established the absolute configuration of all the stereogenic carbons in dactyloditerpenol acetate (**1**) as 1*S*, 6*S*, 7*R*, 10*S*, and 11*R*. Finally, when a comparison was made between the NMR spectroscopic data of dactyloditerpenol acetate (**1**) with those reported for **4**, an unnatural stereoisomer of laurenditerpenol (**2**) prepared by diastereoselective synthesis, the two sets of chemical shift values for comparable centers were almost identical.⁶

Upon screening for cancer cell cytotoxicity against A2058 melanoma and DU-145 prostate cancer cell lines, dactyloditerpenol acetate (**1**) displayed weak activity as the observed reduction for cell viability was only 67% and 77%, respectively. When tested for *in vitro*

antituberculosis activity, compound **1** was found to inhibit growth of *Mycobacterium tuberculosis* H₃₇Rv marginally (MIC 59.4 µg/mL). In contrast, as shown in Fig. 4, dactyloditerpenol acetate (**1**) potently inhibited both TXB₂ generation (IC₅₀ 0.4 µM), and O₂⁻ release (IC₅₀ 1.0 µM), with minimal effect on short-term *in vitro* toxicity (LDH₅₀ > 10 µM). Thus, in our experimental conditions, significant inhibition of LPS-activated rat brain neonatal microglia TXB₂ and O₂⁻ release generation appeared to result from a pharmacologic rather than a toxic effect of compound **1** on microglia *in vitro*. Interestingly, compound **1**, appears more potent than acetylsalicylic acid (aspirin) (IC₅₀ 3.12–10 µM),²⁵ and of similar potency to flurbiprofen (apparent IC₅₀ 100 nM),²⁵ two U.S. Food and Drug Administration approved nonsteroidal anti-inflammatory drugs used to treat pain, fever, and inflammation. Thus, our current data support further development of **1** as a novel anti-neuroinflammatory agent designed to modulate activated brain microglia release of TXB₂ and O₂⁻, both inflammatory mediators associated with neuroinflammation.^{10b}

Compound **1** appears to be biogenetically related to laurenditerpenol (**2**), this in spite of the fact that the latter metabolite is an irregular diterpene with the opposite 1,6-*syn*-3-methylcyclohex-2-en-1-ol ring system.⁵ As *Aplysia dactylomela* frequently grazes upon several *Laurencia* species, it is therefore envisioned that **1** is most likely of dietary origin and that it may actually be a biosynthetically-related *Laurencia* secondary metabolite.²⁶ Thus, dactyloditerpenol acetate could be derived from “regular” head-to-tail arrangement of four isoprene units followed by bromine-assisted cyclization of a suitable diterpene precursor and subsequent oxidation.^{2b} It is not unlikely that the sea hare further undertakes the acetylation of an ingested algal-derived alcohol.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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11. **Animal material and isolation of dactyloditerpenol acetate (1):** forty specimens of the tropical sea hare *Aplysia dactylomela* (Rang, 1828) (subclass Opisthobranchia; order Anaspiidea; superfamily: Aplysioidea; family Aplysiidae) were collected by hand at depths of 1–2 ft off Mona Island, Puerto Rico in June 2011. The organisms were frozen and lyophilized prior to extraction. The dry specimens (137.4 g) were cut into small pieces and blended in a mixture of 1:1 CHCl₃–MeOH (4 L). After filtration, the crude extract was concentrated and stored under vacuum to yield a dark green oil (40 g), which was later suspended in H₂O and extracted with *n*-hexane (4 × 750 mL), CHCl₃ (3 × 1 L), and EtOAc (3 × 800 mL). Concentration of the *n*-hexane solubles yielded a green oil (17 g), a small portion of which (5 g) was further chromatographed over silica gel (60 g) using mixtures of *n*-hexane–EtOAc of increasing polarity (0–100%) and MeOH–EtOAc (1:1). Twelve fractions (I–XII) were generated on the basis of TLC and ¹H NMR analyses. Fraction X (390 mg) was further purified through a silica gel (10 g) column eluting with mixtures of *n*-hexane–EtOAc of increasing polarity (9.5:0.5; 9:1; 8.5:1.5; 8:2; 1:1; 0:1) and MeOH–EtOAc (1:1) to give five sub-fractions (J1–J5). Lastly, sub-fraction J3 (50 mg) was passed through a short plug of silica gel (1.0 g) using a step gradient of *n*-hexane–EtOAc (10:0; 9:1; 8:2) to yield pure dactyloditerpenol acetate (1) (19 mg, 0.05%).
12. Inexplicably, the formation of small white dots was observed during cell cultures and thus, percent viability could not be determined.
13. *Dactyloditerpenol acetate* (1). Colorless oil; [α]_D²⁰ + 7.0 (c 1.4, CHCl₃); IR (film) $\nu_{\max}$ 3418, 2963, 2927, 1735, 1717, 1676, 1452, 1376, 1241, 1025, 982 cm⁻¹; HRESI-MS *m/z* 389.2664 [M + Na]⁺ (calcd for C₂₂H₃₈O₄Na, 389.2668); ¹H NMR (500 MHz, CDCl₃) see Table 1; ¹³C NMR (125 MHz, CDCl₃) see Table 1.
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20. With the aim of strengthening the assignment of relative stereochemistry at the C-7 stereogenic center in dactyloditerpenol acetate (**1**), we undertook a study of the configuration by an integrated QM-NMR approach based on DFT calculations of ^{13}C NMR chemical shifts and the analysis of the experimentally measured NMR spectroscopic data. Critically, ^{13}C NMR chemical shifts were used to validate the theoretical models, and dipolar coupling correlations derived from 2D-NOESY NMR experiments were used to corroborate the arrangements suggested by QM methods and to determine the relative configuration of the molecule. Because all the proton and carbon values had been confidently assigned by 2D-NMR experiments (Table 1) and were in agreement with the proposed structure **1**, we confined our study to the two possible diastereomers based on C-7 (see diastereomer models **1a** and **1b** in Fig. 5 in Supplementary data), the most flexible and thus potentially compromising stereocenter in dactyloditerpenol acetate. To determine the relative configuration of this center the ^{13}C NMR isotropic shifts of each diastereomer (Fig. 5) at its lowest-energy configuration were calculated. The minimum-energy configuration for each isomer was identified by a Monte Carlo conformational search with the MMFF force field as implemented in the Spartan 04 software package. All diastereomers were further optimized at the HF/6-31G(2d,p) level of theory with the aid of the Gaussian 03 program. NMR chemical shifts were calculated by the GIAO method at the mPW1PW91/6-31G(2d,p) level of theory. The experimentally measured values were plotted against the calculated shifts, and a least-squares fit line was determined. The calculated shifts for each isomer were corrected by using the slope and intercept to give scaled ^{13}C NMR chemical shifts. The difference plots were determined by subtracting the corrected shifts from the experimentally determined ones. Diastereomer **1b** presented the best ^{13}C NMR chemical shift correlation with the theoretical values with an average of $\delta = 3.1$ ppm (Table 4). It also resulted in the conformation with the lowest energy relative to the alternate stereoisomer **1a**.
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22. *Derivative 3*. A mixture of **1** (7.0 mg, 0.019 mmol) and anhydrous KCN (1.0 mg, 0.015 mmol) in dry MeOH (5 mL) was stirred for 17 h at 25 °C. The reaction mixture was concentrated *in vacuo* and the oily residue obtained was passed through a short plug of silica gel (0.8 g) eluted with a gradient of *n*-hexane–EtOAc (9:1; 8:2; 7:3) to afford pure compound **3** (1.5 mg, 26%) as a colorless oil. Data for **3**: $[\alpha]_{\text{D}}^{20} + 4.5$ (c 0.5, CHCl_3); UV (CH_3OH) λ_{max} (log ϵ) 202 (4.24) nm; IR (film) ν_{max} 3421, 2962, 2926, 2858, 1515, 1457, 1376, 1262, 1070, 817 cm^{-1} ; ^1H NMR (CDCl_3 , 500 MHz) δ 7.10 (2H, br d, $J = 8.2$ Hz, H-2/H-4), 7.07 (2H, br d, $J = 8.0$ Hz, H-1/H-5), 5.10 (1H, br t, $J = 7$ Hz, H-14), 3.30 (1H, br d, $J = 10$ Hz, H-10), 2.66 (1H, m, H-7), 2.32 (3H, s, H₃-20), 2.10–2.00 (2H, m, H₂-13), 1.32 (2H, m, H₂-8), 1.68 (3H, s, H₃-17), 1.60 (3H, s, H₃-16), 1.86–1.56 (2H, m, H₂-12), 1.36–1.28 (2H, m, H₂-9), 1.24 (3H, d, $J = 6.9$ Hz, H₃-19), 1.10 (3H, s, H₃-18); ^{13}C NMR (CDCl_3 , 125 MHz) δ 144.4 (C-6), 135.3 (C-3), 132.0 (C-15), 129.1 (C-2/C-4), 126.8 (C-1/C-5), 124.5 (C-14), 78.9 (C-10), 74.8 (C-11), 39.7 (C-7), 35.6 (C-12),* 35.5 (C-8),* 29.5 (C-9), 25.7 (C-17), 23.5 (C-18), 22.5 (C-19), 22.1 (C-13), 21.0 (C-20), 17.7 (C-16) (*interchangeable assignments); LREI-MS m/z [$\text{M}]^+$ 304 (1), 286 (2), 204 (21), 185 (13), 159 (19), 145 (14), 132 (100); HREI-MS m/z [$\text{M}]^+$ 304.2405 (calcd for $\text{C}_{20}\text{H}_{32}\text{O}_2$, 304.2402).
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Figure 1. Spin systems deduced through the COSY (bold lines) and key $^{2,3}J$ H $\rightarrow$ C correlations exhibited by the HMBC spectrum of compound **1**.

Figure 2.
Key NOESY correlations for the energy-minimized structure of dactyloditerpenol acetate (1).

Figure 3.
Empirical rules for the determination of absolute configuration of secondary and tertiary alcohols within dactylo diterpenol acetate (1).

Figure 4.

Differential effects of compound **1** on PMA (1 μM)-stimulated O₂⁻, TXB₂ and LDH generation by *E. coli* LPS-activated rat neonatal microglia. The O₂⁻, TXB₂ and LDH assays, and the statistical analysis of the data, were performed as described in reference 27. Data corresponds to three independent experiments. ** P< 0.01, *** P<0.001.

Scheme 1.
Synthesis of derivative **3**.

Table 1¹³C (125 MHz), ¹H NMR (500 MHz), and long-range correlation data for dactyloditerpenol acetate (**1**)^a

N°	δ _C , type ^b	δ _H (mult., J in Hz) ^c	HMBC ^d
1	69.0, CH	4.00 (br s)	2, 3, 6, 7
2	125.6, CH	5.37 (br s)	1, 4, 6, 20
3	137.3, C		
4	30.3, CH ₂	1.98–1.85 (br m)	2, 3, 6
5	20.9, CH ₂	1.57 (m) 1.26 (m)	1, 6
6	46.8, CH	1.26 (m)	1, 4, 19
7	31.1, CH	1.89 (m)	1, 5, 6, 8, 9, 19
8	31.7, CH ₂	1.26–1.24 (br m)	7, 10, 19
9	27.1, CH ₂	1.64–1.60 (br m)	10
10	79.2, CH	4.85 (dd, 9.7, 3.5)	8, 9, 11, 12, 18, 21
11	74.1, C		
12	37.6, CH ₂	1.54 (ddd, 16.4, 11.1, 5.5) 1.43 (ddd, 16.8, 11.1, 5.8)	10, 11, 13, 14
13	22.0, CH ₂	2.12–2.02 (br m)	12, 14, 15
14	124.1, CH	5.09 (br t, 7.1)	12, 13, 16, 17
15	132.1, C		
16	17.6, CH ₃	1.61 (br s)	14, 15, 17
17	25.7, CH ₃	1.68 (br s)	14, 15, 16
18	23.5, CH ₃	1.15 (s)	10, 11, 12
19	14.2, CH ₃	0.79 (d, 6.9)	6, 7, 8
20	23.1, CH ₃	1.66 (br s)	2, 3, 4
21	171.2, C		
22	21.1, CH ₃	2.10 (s)	21

^aSpectra recorded in CDCl₃ at 25 °C.^bChemical shifts refer to CDCl₃ (δ_C = 77.0). Carbon types were determined from DEPT NMR experiments.^cChemical shifts refer to CDCl₃ (δ_H = 7.26).^dHMBC experiment recorded with a delay of 50 ms.

Table 2

Assignment of absolute configuration of dactyloditerpenol acetate (**1**) at 15 mol% per OH of the (*R*)- and (*S*)-Eu(tfc)₃^a

center	$\frac{\delta_R = \delta CX_R - \delta CY_R}{\delta_R}$		$\frac{\delta_S = \delta CX_S - \delta CY_S}{\delta_S}$		$\delta = \delta_R - \delta_S$		
	δCX_R	δCY_R	δCX_S	δCY_S			
C-1	47.31	125.64	-78.33	47.28	125.77	-78.49	+0.16
C-11	80.55	37.78	42.77	80.57	37.76	42.81	-0.04

^a¹³C NMR data for **1** collected in CDCl₃ solution.