Published in final edited form as:

Tetrahedron Lett. 2012 December 5; 53(49): 6641–6645. doi:10.1016/j.tetlet.2012.09.068.

Dolabriferols B and C, non-contiguous polypropionate esters from the tropical sea hare *Dolabrifera dolabrifera*

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Abstract

The Caribbean mollusc *Dolabrifera dolabrifera* from Puerto Rico contains two new propionate-derived metabolites, dolabriferol B and C (**2** and **3**), in addition to the known compound dolabriferol (**1**). The structures of dolabriferol B (**2**) and C (**3**) were established by comparison of their spectral data with those of **1**, and the absolute configuration of **2** was determined from chemical degradation studies. The structure of dolabriferol C (**3**) was confirmed by X-ray analysis.

Keywords

Polypropionate esters; *Dolabrifera dolabrifera*; X-Ray analysis; Cytotoxicity; Tuberculosis

For nearly half a century, marine natural product chemists have shown that gastropod molluscs (Opisthobranchs) are an abundant source of secondary metabolites derived from the iterative condensation of propionate units.¹ Yet, a small number of these metabolites do not contain a contiguous carbon framework as predicted from regular polyketide biosynthesis.² Dolabriferol (**1**), isolated in 1996 by Gavagnin and co-workers from the anaspidean mollusc *Dolabrifera dolabrifera*, is based on a non-contiguous polypropionate backbone and thus, is considered a prototypical example of this rare class of natural products.³ After its initial discovery, dolabriferol (**1**) quickly became the object of intense scrutiny of organic synthetic chemists.⁴ Hitherto, no relevant biological activity data for any member of this interesting class of compounds have been described.

As part of our ongoing efforts to discover biologically active natural products from Caribbean marine organisms, fresh specimens of *Dolabrifera dolabrifera* from Puerto Rico were chemically investigated.⁵ In this paper we report the isolation of dolabriferol (**1**) along with two new non-contiguous polypropionate esters, dolabriferol B (**2**)⁶ and dolabriferol C (**3**),⁷ whose structures have been proposed on the basis of the interpretation of their spectroscopic data. Taking into account that all attempts by Gavagnin and coworkers to establish the absolute configuration of dolabriferol (**1**) by chemical derivatization at C6 failed,³ with new material in hand, we followed a different approach. Upon measuring

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Supplementary data

Supplementary data associated with this article (Photographs of *D. dolabrifera*, experimental procedures, Table 3, Fig. 4, and copies of the 1D and 2D NMR spectra for compounds **2** and **3** can be found, in the online version, at <http://dx...>

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the ^{13}C NMR chemical shift of the carbons adjacent to the secondary alcohol in the presence of chiral lanthanide shift reagents, the absolute configuration at C6 in **1** was found to be *R* (see Supplementary data).⁸ With the relative configuration of **1** firmly established by the original investigators by X-ray crystallographic analysis, we found the absolute configuration of **1** to be 4*S*, 6*R*, 7*R*, 12*R*, 13*S*, 15*S*, 16*S*, and 18*S*. Our results confirm a recent determination by Vogel and co-workers of the absolute configuration of (–)-dolabriferol (**1**) by total synthesis.^{4e}

Dolabriferol B (**2**) was isolated as an optically active ($[\alpha]_{\text{D}}^{20} -51.9^\circ$) colorless oil, whose molecular formula, established by HR-ESIMS to be $\text{C}_{20}\text{H}_{36}\text{O}_6$, differed by one carbon and two hydrogens less than that of **1**. The IR spectrum showed a broad band at 3425 cm^{-1} (OH's) as well as two bands ascribable to carbonyl functionalities (1721 and 1719 cm^{-1}). Analysis of ^{13}C and DEPT-135 NMR spectra (Table 1) indicated the presence in **2** of 7 methyl, 3 methylene, 7 methine, and 3 non-protonated carbons, two of which were assigned to carbon atoms for saturated ketone (δ 215.3) and ester (δ 173.6) carbonyls and one was a hemiketal carbon (δ 98.6).

The ^1H NMR spectrum of **2** showed the presence of three oxymethine protons at δ 3.76 (dt, $J = 10.0, 5.0\text{ Hz}$), 5.23 (dd, $J = 2.7, 2.5\text{ Hz}$), and 3.69 (ddd, $J = 10.7, 8.0, 3.0\text{ Hz}$) assigned to H-6, H-15, and H-18, respectively, by analysis of ^1H - ^1H COSY, HSQC, and HMBC spectra as well as by comparison with data obtained for dolabriferol (**1**).³ ^1H - ^1H COSY and HSQC experiments quickly allowed us to pinpoint signals attributable to three ethyl groups: two isolated (2.45 – 2.57 , H₂-2; 1.04 , H₃-1 and 1.59 – 1.67 , H₂-10, 0.93 , H₃-11) and one (1.39 – 1.67 , H₂-19; 0.94 , H₃-20) further linked to H-18 (δ_{H} 3.69). The presence of four methyl doublets at δ 0.80, 1.00, 1.15, and 1.32 (with J s $\sim 7.0\text{ Hz}$) and two hydroxyl groups (indicated by two D₂O exchangeable signals at δ 3.66 and 3.58) in the ^1H NMR spectrum established with confidence the polypropionate origin of **2**. Side-by-side comparisons of the NMR spectra of **2** with those for dolabriferol (**1**) revealed striking similarities suggesting nearly identical structures (see Table 3 in Supplementary data). Indeed, detailed analysis of the ^1H - ^1H COSY, HSQC, and HMBC spectra of **1** (Table 1 and Fig. 1) showed the presence of many of the same partial structural units in the molecule as those in **1**. The only significant variations in the ^1H NMR spectrum between compounds **2** and **1** were the disappearance of the signals due to an isopropyl group ascribable in **1** to H₃-20 (δ_{H} 1.00, 3H, d, $J = 6.8\text{ Hz}$) and H₃-21 (δ_{H} 0.83, 3H, d, $J = 6.8\text{ Hz}$) and the appearance of a new triplet signal in **2** at δ_{H} 0.94 (3H, $J = 7.4\text{ Hz}$), as well as the subtle shifts in the protons H-16 (δ_{H} 1.68) and H-18 (δ_{H} 3.69). These data suggested that the modifications in this compound involved the alkyl side chain appended to the ring moiety of **2** at C18. The connectivities observed in ^1H - ^1H COSY, HSQC, and HMBC experiments made it possible to assign the carbons about the heterocyclic ring (C10→C20) as follows: δ_{C} 32.4 (CH₂, C10), 7.1 (CH₃,

C11), 98.6 (C, C12), 39.6 (CH, C13), 12.8 (CH₃, C14), 76.5 (CH, C15), 38.3 (CH, C16), 13.2 (CH₃, C17), 69.9 (CH, C18), 25.2 (CH₂, C19) and 8.9 (CH₃, C20). Thus, we concluded that all of these spectroscopic variations can only be explained by the presence in **2** of an ethyl moiety at C18. The remaining ¹H and ¹³C NMR signals in CDCl₃ assigned to the polypropionate ester side chain of **2** were essentially identical to those of **1**.

The relative configuration about the cyclic hemiketal in **2** was assigned by proton coupling constant analysis and a NOESY NMR experiment. The large $J_{16,18}$ coupling constant (10.7 Hz) between H16 and H18 placed these two protons in a *trans* diaxial relationship suggesting that the pyranoid ring adopts a thermodynamically preferred chair conformation with the C16 and C18 substituents in equatorial positions. The smaller $J_{13,15}$ and $J_{15,16}$ coupling constants (2.7 and 2.5 Hz, respectively) supported a *gauche* arrangement between H13 and H15 and between H15 and H16. The observed $J_{13,14}$ and $J_{16,17}$ coupling constants of 7.1 and 7.0 Hz, respectively, for the C14 and C17 methyl groups, placed them in equatorial positions about the THP ring.⁹ In addition, steric interactions were observed between the proton at δ 5.23 (H15) and those resonating at δ 1.93 (H13), 1.68 (H16), 1.00 (H₃-14), and 0.80 (H₃-17), confirming the relative configuration of H15. The configuration at C12 in **2** was established conclusively from strong NOESY correlations of 12-OH with H₃-14 and H18, thus arguing for the contention that the hydroxyl group at C12 was fixed with a donor oxygen nearby in a H-bond. Such constraint requires a *cis* arrangement of the oxygen bearing substituents at both C12 and C15, thereby establishing a C12*R** configuration.¹⁰ The relative configuration of C4, C6, and C7 in **2** was assumed to be the same as in **1** based on the fact that the spectral and optical rotation properties of **1**, $[\alpha]_D^{20}$ -53.0° (*c* 1.1, CHCl₃), are in close agreement with those of **2**, $[\alpha]_D^{20}$ -51.9° (*c* 0.7, CHCl₃).¹¹ Furthermore, as **1** and **2** slowly decompose into the same degradation product **4**, we herein propose the absolute configuration of **2** as 4*S*, 6*R*, 7*R*, 12*R*, 13*S*, 15*S*, 16*S*, and 18*S*.¹²

Dolabriferol C (**3**) was obtained as an optically active, white crystalline solid ($[\alpha]_D^{20}$ -45.2° (*c* 0.7, CHCl₃)). The molecular formula for **3** was determined as C₂₇H₄₈O₈ from its HR-ESIMS quasimolecular $[M + Na]^+$ ion observed at m/z 523.3238, six carbon, ten hydrogen and two oxygen atoms (C₆H₁₀O₂) more than the formula of dolabriferol (**1**). The IR spectrum showed absorbance consistent with hydroxyl ($\nu_{\max}$ 3452 cm⁻¹) and carbonyl ($\nu_{\max}$ 1731 and 1713 cm⁻¹) functions. The ¹³C NMR data for this compound provided 27 carbon signals due to two ketone and one ester carbonyl groups, one acetal quaternary carbon, eleven sp³ methines including four oxymethines, two sp³ methylenes, and ten methyl groups, suggesting a polypropionate skeleton (Table 2). Five isolated spin systems were established from analysis of the ¹H-¹H COSY and HSQC experiments: (a) two of them involved ethyl groups, H₃-1 (δ_H 1.00)/H₂-2 (δ_H 2.55) and H₃-17 (δ_H 0.97)/H₂-16 (δ_H 1.72), (b) two were systems connecting five carbons, from methyl H₃-5 (δ_H 1.09) to methyl H₃-8 (δ_H 1.10) and from methyl H₃-11 (δ_H 1.01) to methyl H₃-14 (δ_H 1.37), and (c) a system consisting of nine carbons, from methyl H₃-20 (δ_H 1.06) to a pair of geminal methyls H₃-26 (δ_H 1.01) and H₃-27 (δ_H 0.84) (Fig. 2). The fragments C1-C15 and C16-C27 were deduced by ¹H-¹H COSY, HSQC and HMBC data by comparison with the spectroscopic

data for dolabriferol (**1**). Comparison of the NMR (^1H and ^{13}C) data for **3** with those for **1** demonstrated that most of these data were virtually identical except for some changes associated with the polypropionic side chain. From these data it was inferred that dolabriferol C (**3**) was likely a homolog of **1** with an elongated acyclic chain. The spectroscopic data for the tetrahydropyranyl ring fragment were again assigned by analysis of 2D spectra, in particular the COSY correlations from H19 through to H25 and associated methyls, which matched closely the equivalent data for **1**. The chemical shift of H21 (δ 5.24) and HMBC data implied that the ester moiety was likely attached through the same position as **1**.

The relative configuration in the pyranoid ring and that at C10 and C13 in compound **3** was established as identical with that observed for compounds **1** and **2** based on biogenetic grounds and by comparing their NMR data and NOE correlations observed in the NOESY experiment (Table 2). Compound **3** may be derived from **1** thus suggesting that the stereochemistry of these compounds at the equivalent chiral centers must be the same.¹³ In order to confirm the proposed structure and to establish unambiguously the relative configuration at C4, C6, and C7 along the C₁₅ acyclic chain in dolabriferol C (**3**), a single-crystal X-ray diffraction analysis was performed.

Recrystallization of **3** by slow evaporation from a 95:5 mixture of *n*-hexane/EtOAc gave crystals of excellent quality that were amenable to X-ray crystallographic analysis. The X-ray structure, which defines only the relative configuration, is shown in Fig. 3.¹⁴ Hence, the overall relative configuration for the eleven stereocenters of dolabriferol C was assigned as 4*R**, 6*S**, 7*R**, 10*S**, 12*R**, 13*R**, 18*R**, 19*S**, 21*S**, 22*S**, and 24*S**. The assigned configurations, which were in full agreement with the correlations observed in the NOESY spectrum of **3**, were confirmed through interpretation of NMR coupling data. An attempted Mosher's esterification of **3** resulted in a mixture of unreacted starting material suggesting that the strong intramolecular hydrogen bonds between the hydroxyl protons and carbonyl oxygens impeded the esterification reaction. Sadly, we were unable to retrieve the unreacted polypropionate which prevented any further attempts at resolving the absolute configuration of this compound. Yet, the $[\alpha]_D$ values of **3** (-45.2°) and **1** (-53.0°) suggest that the absolute configuration of dolabriferol C (**3**) at the analogous asymmetric carbons is probably identical to that of dolabriferol (**1**) since their specific rotations are similar both in sign and order of magnitude and because in both instances the optical rotation might be mainly attributed to the same isolated pyranoid ring moiety.

Biological screening of compounds **1–3** indicated no significant *in vitro* A2058 melanoma or DU145 prostate cancer cell cytotoxicity at 10 μM (cells treated in a dose-dependent manner with these compounds showed viabilities ranging from 81–97%). Follow up biological screening revealed that at a concentration of 128 $\mu\text{g/mL}$ compounds **2** and **3** effect inhibitory activity (39% and 93%, respectively) against *Mycobacterium tuberculosis* H37Rv. Unfortunately, the paucity of compounds **1–3** that we had in hand combined with the instability of **1** and **2** prevented attempts to acquire additional biological activity data. We anticipate that the solution to the supply problem will be more easily accomplished by synthesis than attempting to re-isolate these metabolites.⁴ Still, compounds **2** and **3** represent welcome additions to a very short list of non-contiguous polypropionate esters isolated from marine organisms.² Indeed, dolabriferol C (**3**) is apparently the first ester obtained by coupling an intact C₁₅ polypropionate acid to a propionate alcohol. Although they could be classified as natural products, the fact that compounds **1–3** were isolated in meager quantity suggests that they might be artifacts from rearrangement during storage or workup. The isolation of propionate-derived metabolites following routine extraction methods and facile interconversion under mild conditions to non-continuous polypropionates, has been previously remarked.¹⁵

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We thank Y. M. Pérez and M. Dorado for collecting the mollusc and Dr. R. G. Raptis (UPR-RP) for access to his X-ray crystallographic facilities. Extracts were screened for anti-tumor and anti-TB activity by Dr. S. Nam (City of Hope Beckman Research Institute) and Dr. Y. Wang (Institute for Tuberculosis Research of the University of Illinois at Chicago), respectively. HR-ESIMS data were provided by the Mass Spectrometry Laboratory of the University of Illinois at Urbana-Champaign. Financial support to C. J.-Romero was provided by the IFARHU-SENACYT Program of the Republic of Panama. This work was supported by NIH-SC1 Grant 1SC1GM086271-01A1 awarded to A.D.R.

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5. *Collection, Extraction and Isolation*. Eight specimens of the tropical sea hare *Dolabrifera dolabrifera* (Rang, 1828) (subclass Opisthobranchia; order Anaspidea; family Plakinidae) were collected by hand at depths of 1–2 ft off El Escambrón, Puerto Rico, in January 2011. The organism was frozen and lyophilized prior to extraction. The dry specimens (14 g) were cut into small pieces and left steeping in 1:1 CHCl₃–MeOH (350 mL) for 48 h. After filtration, the crude extract was concentrated to yield a dark green oil (600 mg), which was later suspended in H₂O and extracted with *n*-hexane (3 × 250 mL) and EtOAc (3 × 250 mL). Concentration of the *n*-hexane solubles yielded an oil (405 mg) that was further chromatographed over Si gel (8.0 g) using mixtures of *n*-hexane–EtOAc of increasing polarity (0–100%). Fraction VII(F) (27 mg) was passed through a short plug of Si gel (1.0 g) using a step gradient of *n*-hexane–EtOAc (10:0; 9.8:0.2; 9.6:0.4; 9.4:0.6; 9.2:0.8; 9:1) to yield dolabriferol (**1**) (4.3 mg, 0.7%), dolabriferol B (**2**) (2.1 mg, 0.35%), and dolabriferol C (**3**) (2.4 mg, 0.4%).
6. *Dolabriferol B* (**2**). Colorless oil; [α]_D²⁰ –51.9 (*c* 0.7, CHCl₃); IR (film) $\nu_{\max}$ 3425 (br), 2969, 2939, 2881, 1721, 1719, 1461, 1378, 1277, 1218, 1125, 1039, 984 cm^{–1}; ¹H NMR (CDCl₃, 500 MHz) and ¹³C NMR (CDCl₃, 125 MHz) (see Table 1); HR-ESIMS *m/z* [M+Na]⁺ calcd for C₂₀H₃₆O₆Na 395.2410, found 395.2417.
7. *Dolabriferol C* (**3**). White crystalline solid; [α]_D²⁰ –45.2 (*c* 0.7, CHCl₃); IR (film) $\nu_{\max}$ 3452 (br), 2969, 2937, 2880, 1731, 1713, 1461, 1378, 1220, 1126, 1033, 983 cm^{–1}; ¹H NMR (CDCl₃, 500 MHz) and ¹³C NMR (CDCl₃, 125 MHz) (see Table 2); HR-ESIMS *m/z* [M+Na]⁺ calcd for C₂₇H₄₈O₈Na 523.3247, found 523.3238.
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9. The coupling constant for an equatorial methyl group, on a tetrahydropyran (THP) ring, is generally 7 Hz, see: Hochlowski JE, Coll JC, Faulkner DJ, Biskupiak JE, Ireland CM, Qi-tai Z,

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10. Indeed, the presence of intramolecular hydrogen bonding in dolabriferol B (**2**) was supported experimentally by the strong couplings of H6 and 6-OH ($J = 10.0$ Hz) and the strong NOEs detected between 12-OH and H8, H14, and H18.
 11. The optical rotation we measured for **1** ($[\alpha]_D = -53.0^\circ$) is notably higher than that reported in ref. 3 ($[\alpha]_D = -29.4^\circ$).
 12. Prolonged exposure of esters **1** and **2** to air in CHCl_3 solution at 20°C led to acid **4** within days. *Compound 4* (1.5 mg): colorless oil; $[\alpha]_D^{20} -38.9$ (c 0.9, CHCl_3); IR (film) ν_{max} 3460 (br), 2976, 2940, 1714 (br), 1461, 1379, 1192, 1111, 1031, 975 cm^{-1} ; ^1H NMR (CDCl_3 , 500 MHz) δ 3.79 (1H, dd, $J = 6.8, 5.2$ Hz, H-6), 2.86 (1H, dq, $J = 7.1, 6.8$ Hz, H-4), 2.70 (1H, dq, $J = 7.1, 5.2$ Hz, H-7), 2.61 (1H, dq, $J = 14.4, 7.2$ Hz, H-2 α), 2.49 (1H, dq, $J = 14.4, 7.2$ Hz, H-2 β), 1.29 (3H, d, $J = 7.1$ Hz, Me-8), 1.20 (3H, d, $J = 7.1$ Hz, Me-5), 1.06 (3H, t, $J = 7.2$ Hz, Me-1); ^{13}C NMR (CDCl_3 , 125 MHz) δ 216.3 (C, C3), 178.4 (C, C9), 75.8 (CH, C6), 48.5 (CH, C4), 42.3 (CH, C7), 35.7 (CH₂, C2), 14.6 (CH₃, C8), 14.5 (CH₃, C5), 7.4 (CH₃, C1); HR-ESIMS m/z $[\text{M}+\text{Na}]^+$ calcd for $\text{C}_9\text{H}_{16}\text{O}_4\text{Na}$ 211.0946, found 211.0955.
 13. Since dolabriferol C (**3**) very likely is formed by propionate elongation of dolabriferol (**1**), it must have one of four possible diastereoisomeric structures. A distinction between these structures is possible by a combination of coupling constant analysis, NOESY, and molecular modeling studies. We used the MacSpartan '04 molecular modeling program to calculate the conformer distribution for each possible structure; key interproton distances and dihedral angles were then measured on the lowest energy conformer. After careful evaluation of all of the data gathered, the observed NOESY correlations and coupling constants in CDCl_3 (Table 2) were found to be consistent only with structure **3**. Our data clearly suggest the preferential population of a single local backbone conformation along the ten bonds between C1 and C15 of the acyclic chain that was supported by the formation of intramolecular hydrogen bonds. A model for the preferred conformation, interproton distances and J data are given as Supplementary data (Fig. 4), together with selected observed NOESY correlations (for simplicity's sake, only the values pertaining to the C15 polypropionate chain of **3** are illustrated). Not surprisingly, in the final crystallographic model depicted in Fig. 3, the side-chain of dolabriferol C (**3**) is bent differently.
 14. Crystallographic data (excluding structure factors) for dolabriferol C (**3**) have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication no. CCDC 890409. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK, (fax: +44-(0)1223-336033 or e-mail: deposit@ccdc.cam.ac.uk).
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Figure 1. Substructures developed for compound **2** on the basis of COSY (bold line) and HMBC ($H \rightarrow C$) correlations.

Tetrahedron Lett. Author manuscript; available in PMC 2013 December 05.

Figure 3.
A perspective view of the final crystallographic model of dolabriferol C (**3**).

Table 1

¹H NMR (500 MHz), ¹³C NMR (125 MHz), ¹H-¹H COSY, HMBC, and NOESY spectroscopic data for dolabriferol B (2) in CDCl₃ at 25 °C^a

atom	δ _C	mult ^b	δ _H , mult (J in Hz)	¹ H- ¹ H COSY	HMBC ^c	NOESY
1	7.4	CH ₃	1.04, t (7.2)	2αβ	2αβ	4
2α	35.9	CH ₂	2.57, dq (18.1, 7.2)	1, 2β	1	
2β			2.45, dq (18.1, 7.2)	1, 2α		
3	215.3	C			1, 2αβ, 4, 5, 6	
4	49.3	CH	2.79, dq (7.1, 5.0)	5, 6	5, 6, 6-OH	1, 6
5	14.4	CH ₃	1.15, d (7.1)	4	4	6
6	75.6	CH	3.76, dt (10.0, 5.0)	4, 6-OH, 7	4, 5, 6-OH, 8	4, 5, 7, 8
7	43.7	CH	2.73, dq (7.1, 5.0)	6, 8	4, 6, 6-OH, 8	6
8	15.5	CH ₃	1.32, d (7.1)	7	6, 7	6, 12-OH, 17
9	173.6	C			6, 7, 8, 15	
10α	32.4	CH ₂	1.59, dq (14.5, 7.6)	10β, 11	11, 12-OH	
10β			1.67, m	10α, 11		13
11	7.1	CH ₃	0.93, t (7.6)	10αβ	10αβ	
12	98.6	C			10αβ, 11, 12-OH, 13, 14, 15, 18	
13	39.6	CH	1.93, dq (7.1, 2.7)	14, 15	10β, 12-OH, 14, 15, 16	10β, 15
14	12.8	CH ₃	1.00, d (7.1)	13	13, 15	6-OH, 12-OH, 15
15	76.5	CH	5.23, dd (2.7, 2.5)	13, 16	14, 17	13, 14, 16, 17
16	38.3	CH	1.68, m	15, 17, 18	15, 17	15
17	13.2	CH ₃	0.80, d (7.0)	16	15, 16	6-OH, 8, 15, 18
18	69.9	CH	3.69, ddd (10.7, 8.0, 3.0)	16, 19αβ	15, 16, 17, 19αβ, 20	12-OH, 17, 20
19α	25.2	CH ₂	1.39, dq (14.4, 7.4)	18, 19β, 20	20	
19β			1.67, m	18, 19α, 20		
20	8.9	CH ₃	0.94, t (7.4)	19αβ	18, 19αβ	18
6-OH			3.58, br d (10.0)	6		14, 17
12-OH			3.66, br s			8, 14, 18

^aChemical shift values are in ppm relative to the residual CHCl₃ (δ 7.26) or CDCl₃ (δ 77.0) signals.

^b¹³C NMR multiplicities were obtained from a DEPT-135 experiment.

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¹J_{CH} = 6 and 8 Hz.
Protons correlated to carbon resonances in the ¹³C column. Parameters were optimized for 2,3

Table 2

¹H NMR (500 MHz), ¹³C NMR (125 MHz), ¹H–¹H COSY, HMBC, and NOESY spectroscopic data for dolabriferol C (3) in CDCl₃ at 25 °C^a

atom	δ _C	mult ^b	δ _H , mult (J in Hz)	¹ H– ¹ H COSY	HMBC ^c	NOESY
1	7.3	CH ₃	1.00, t (7.3)	2αβ	2αβ	2αβ
2αβ	36.6	CH ₂	2.55, m	1	1	1, 4, 5, 6, 12-OH
3	218.2	C			1, 2αβ, 4, 5, 6	
4	47.0	CH	2.85, dq (7.3, 5.2)	5, 6	5, 6-OH	2αβ, 5, 6, 6-OH, 8, 11
5	14.2	CH ₃	1.09, d (7.3)	4	4, 6	2αβ, 4, 6
6	72.0	CH	4.19, dt (7.0, 5.2)	4, 6-OH, 7	4, 5, 6-OH, 7, 8	2αβ, 4, 5, 6-OH, 7, 8, 10, 12-OH, 20
7	50.3	CH	2.88, dq (6.9, 5.2)	6, 8	8	6, 8, 10, 12-OH, 18-OH
8	9.3	CH ₃	1.10, d (6.9)	7	6, 7	4, 6, 7
9	216.6	C			7, 8, 10, 11	
10	49.8	CH	3.05, dq (10.0, 6.9)	11, 12	11	6, 7, 11, 12-OH, 13, 18-OH, 20
11	14.3	CH ₃	1.01, d (6.9)	10	10	4, 10, 12, 13
12	76.8	CH	3.64, ddd (11.4, 10.0, 3.0)	10, 12-OH, 13	10, 11, 12-OH, 14	11, 12-OH, 13, 14
13	43.2	CH	2.78, dq (7.2, 3.0)	12, 14	14	10, 11, 12, 14, 18-OH
14	16.3	CH ₃	1.37, d (7.2)	13	13	12, 13, 23
15	173.8	C			12, 13, 14, 21	
16αβ	32.8	CH ₂	1.72, m	17	17, 18-OH	18-OH, 19
17	7.2	CH ₃	0.97, t (7.4)	16αβ	16αβ	18-OH, 27
18	98.4	C			16αβ, 17, 18-OH, 19, 20, 21	
19	39.2	CH	1.92, dq (7.1, 2.8)	20, 21	18-OH, 20, 21	16αβ, 20, 21, 22
20	13.1	CH ₃	1.06, d (7.1)	19	19, 21	6, 10, 19, 21
21	76.6	CH	5.24, dd (2.8, 2.6)	19, 22	20, 23	19, 20, 22, 23
22	36.7	CH	1.76, ddd (10.6, 6.9, 2.6)	21, 23, 24	21, 23	19, 23
23	12.9	CH ₃	0.78, d (6.9)	22	21	14, 21, 22, 24, 25
24	72.4	CH	3.68, dd (10.6, 2.3)	22, 25	21, 23, 25, 26, 27	18-OH, 23, 25, 26
25	27.9	CH	1.80, dsp (6.8, 2.3)	24, 26, 27	24, 26, 27	23, 24, 26, 27
26	20.2	CH ₃	1.01, d (6.8)	25	24, 25, 27	24, 25, 27
27	14.0	CH ₃	0.84, d (6.8)	25	24, 25, 26	17, 25, 26

atom	δ_C	mult ^b	δ_H , mult (<i>J</i> in Hz)	¹ H- ¹ H COSY	HMBC ^c	NOESY
6-OH			3.25, br d (7.0)	6		4, 6
12-OH			4.04, br d (11.4)	12		2, 6, 7, 10, 12
18-OH			3.97, br s			7, 10, 13, 16 α β, 17, 24

^aChemical shift values are in ppm relative to the residual CHCl₃ (δ 7.26) or CDCl₃ (δ 77.0) signals.

^b¹³C NMR multiplicities were obtained from a DEPT-135 experiment.

^cProtons correlated to carbon resonances in the ¹³C column. Parameters were optimized for 2,3-¹³CH = 6 and 8 Hz