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Article

Semisynthesis of Dolabellane Diterpenes: Oxygenated Analogues with Increased Activity against Zika and Chikungunya Viruses

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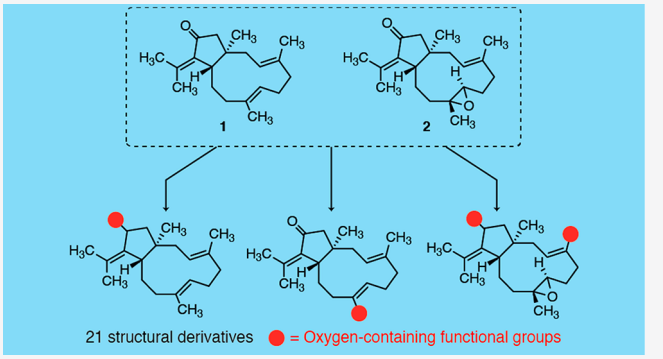
ABSTRACT: Brown algae and soft corals represent the main marine sources of dolabellane diterpenes. The antiviral activity of dolabellanes has been studied for those isolated from algae, whereas dolabellanes isolated from soft corals have been barely studied. In this work, a collection of dolabellane diterpenes consisting of five natural and 21 semisynthetic derivatives was constructed, and their antiviral activities against Zika (ZIKV) and Chikungunya (CHIKV) viruses were tested. Dolabellatrienone (**1**) and (1*R*,7*R*,8*R*,11*S*)-7,8-epoxy-13-keto-dolabella-3,12(18)-diene (**2**), isolated from *Eunicea* genus soft corals, were employed to obtain 21 dolabellane and dolastane diterpenes by reactions such as allylic oxidations, reductions, acid-catalyzed epoxide ring opening, and acetylations. All of the compounds were identified by a combination of one- and two-dimensional NMR, mass spectrometry, and X-ray diffraction experiments. The cytotoxicites against Vero cells and the antiviral activities against ZIKV and CHIKV was tested to calculate the half-maximal effective concentration (EC_{50}) and selectivity indexes (SIs). In general, the addition of oxygen-containing functional groups improved the bioactivity of dolabellane and dolastane diterpenes against ZIKV and CHIKV replication. Compound **9** showed an EC_{50} = 0.92 ± 0.08 μM and SI = 820 against ZIKV.



Arboviruses have caused considerable concern in public health worldwide. Most arboviruses belong to the *Alphavirus* (*Togaviridae* family) and *Flavivirus* (*Flaviviridae* family) genera; other important members relevant to human health belong to the *Bunyaviridae, Reoviridae,* and *Rhabdoviridae* families. This group of RNA viruses exhibits a major genetic plasticity and a high frequency of mutation, which allow the viruses to adapt to both vertebrate and invertebrate hosts.[1] Flavaviviruses are single-stranded ribonucleic acid (RNA) viruses that possess several routes of transmission and cause a variety of symptoms such as hemorrhagic fever and fetal abnormalities.[2] The potential of several synthetic compounds as inhibitors of the viral replication of flavaviruses has been tested in vivo and in vitro.[3] Since the Zika virus (ZIKV) was detected in Brazil in 2015, it has spread explosively across the Americas and has been associated with the increase in cases of microcephaly and Guillain-Barre syndrome (GBS). Although the ZIKV and the Chikungunya virus (CHIKV) share the same mosquito vector and their infections share commonalities, the CHIKV is unique by causing arthritis and arthralgia that may persist for a year or more. In the absence of an effective treatment or vaccines to prevent and control the impact of these viruses, the morbidity and mortality intensifies with considerable implications on health services.[4,5] Therefore, there is a need for compounds to combat, prevent, and control efficiently the impact of these viruses.

Marine algae are an important source of structurally diverse natural products with broad biological activities.[6] Among them, brown algae are a prolific source of dolabellanes (a fused bicyclic [9.3.0] diterpene core) able to inhibit the replication of viruses such as human immunodeficiency virus-1 (HIV-1) and herpes simplex virus-1 (HSV-1).[7−9] So far, dolabellatrienol, a dolabellane isolated from *Dyctiota friabilis*, is one of the most promising antiviral compounds due its capacity to inhibit HIV-1 replication. Further studies indicate that dolabellanetriol's mechanism of action is consistent with a non-nucleoside reverse transcriptase inhibitor (NNRTI).[8,10−12] Another source of diterpenes with different

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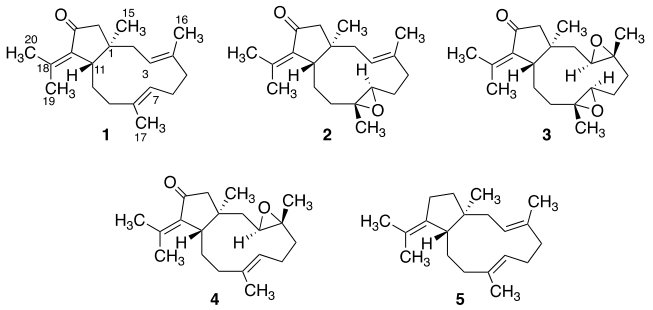
structural arrangements, including other dolabellanes, is soft corals.[13,14] Yet, the potential of soft corals as a source of antiviral dolabellanes has not been deeply explored, as there are only a few studies in this field. Previous work from our research group revealed that dolabellanes isolated from *Eunicea laciniata*, collected at the Colombian Caribbean Sea, inhibited HSV-1 replication, whereas semisynthetic derivatives obtained from dolabellatrienone (**1**) exhibited increased anti-HIV activity.[15,16] A noteworthy difference between dolabellanes obtained from *Eunicea* soft corals and *Dictyota* brown algae is the opposite absolute configuration in the fused bicyclic ring system. These findings suggest that soft corals can also contribute to the discovery of more antiviral dolabellanes, and it is hypothesized that, regardless of the configuration of the bicyclic core, oxygen-containing functional groups are the main factor that influence the antiviral activity of dolabellane diterpenes. Considering that there is not enough evidence to establish a relationship between the structure of oxygenated dolabellanes and their antiviral activity, a chemical library of dolabellanes diterpenes was assembled in this work. The library was developed based on dolabellanes isolated from *Eunicea* soft corals, and special attention was given to test the influence of oxygen-containing groups on the antiviral activity. Semisynthetic derivatives were obtained through straightforward and concise transformations, aiming to include additional alcohols and aldehydes on the dolabellane core. Moreover, the cytotoxicities of the compounds were tested, and the antiviral activity against the replication of ZIKV and CHIKV was also evaluated.

RESULTS AND DISCUSSION

In this work, a collection of dolabellanes with further oxygen-containing functional groups was constructed with the aim of improving their antiviral activities. Compounds **1**−**5** (Chart 1)

Chart 1. Dolabellanes Isolated from *E. laciniata* and *E. asperula* Soft Corals



were previously isolated from *Eunicea* genus soft corals, and their structures were elucidated based on their spectroscopic properties and comparison with literature data.[16] Among them, dolabellatrienone (**1**) and (1*R*,7*R*,8*R*,11*S*)-7,8-epoxy-13-keto-dolabella-3,12(18)-diene (**2**) possess functional groups (e.g., keto groups at C-13, double bonds and epoxides at C-3/C-4 and C-7/C-8) that can be used to include further oxygen-containing functional groups. Furthermore, compounds **1** and **2** were isolated in multigram amounts from the natural source, which is advantageous compared to dolabellanes isolated from brown algae. Summarizing, the different structural features and considerable available amounts of compounds **1** and **2** made them the best candidates to construct a collection of semisynthetic derivatives.

The reactions employed in this work focused on including mainly hydroxy groups, opening of epoxides, and the addition of acetyl and methoxy groups. The elucidation of the structures and the results are discussed by kind of reaction to facilitate the analysis. Compound **3** was not isolated in sufficient quantities from the studied soft corals, but the epoxidation of dolabellatrienone (**1**) with *meta*-chloroperoxybenzoic acid (m-CPBA) to obtain compound **3** was conducted. Employing 2 equiv of m-CPBA, the non-naturally abundant compound **3** is obtained with good yields from the dolabellatrienone (**1**) (Scheme 1).

Allylic Oxidation Reactions. The methyl groups on double bonds in dolabellatrienone (**1**) and compound **2** are reactive toward allylic oxidation, and both compounds were treated under the Riley oxidation conditions (Scheme 1 and Scheme 2). During the course of our experiments, thin-layer chromatography (TLC) analysis showed that the longer the reaction time, the more complex the distribution of reaction products. We hypothesize that the proportion of compounds of higher polarity increases with extended reaction times, and we focused on isolating the major reaction products under the experimental conditions employed (see the Experimental Section).

Dolabellatrienone (**1**) yielded compounds **6** and **7** as major reaction products (Scheme 1). The presence of characteristic signals for an oxygenated methylene in the ^{1}H NMR spectrum at δ_H 4.29 (1H, d, J = 11.2 Hz, H-17a) and δ_H 3.98 (1H, d, J = 11.2 Hz, H-17b) together with the signal at ^{13}C NMR in 59.7 (CH_2, C-17) suggest the presence of a new alcohol in compound **6**. To determine the position of the hydroxy group, a complete analysis by two-dimensional (2D) NMR spectroscopy was conducted as follows. The methyl signal at δ_H 1.42 (s, CH_3-16/δ_C 15.6, CH_3-16) showed heteronuclear multiple bond correlation (HMBC) correlations to a quaternary carbon at δ_C 135.5 (C-4) and a methine at δ_C 125.2 (CH, C-3). The signal for the proton at C-3 δ_H 5.27 (1H, dd, J = 11.2, 5.1 Hz, H-3/δ_c 125.3, CH-3) showed a cross-peak in the homonuclear correlated spectroscopy (COSY) spectrum with protons of a methylene at 2.10 (1H, m, H-2a/δ_c 40.0, CH_2-2) and 1.60 (1H, m, H-2b). In addition, the methyl at δ_H 1.22 (s, CH_3-15) showed an HMBC correlation with H_2-2. In this way, it was determined that C-16 was not oxidized. The remaining olefinic proton at δ_H 5.19 (1H, m, H-7/δ_c 135.0, CH-7) showed HMBC correlations to a methylene at δ_C 34.5 (CH_2-9/δ_H 2.43, 1H, m, H-9a and 2.26, 1H, m, H-9b) and to the diagnostic oxidized methylene at CH_2-17. After an exhaustive study of the NMR spectra, the complete correlations allowed us to correct the structure previously reported for compound **6**, which had an oxidized C-16.[15]

At prolonged reaction times, compound **6** is transformed into compound **7**. The ^{1}H NMR spectrum of compound **7** shows characteristic signals for an α,β-unsaturated aldehyde at δ_H 10.09 (1H, s, H-17) and δ_H 6.40 (1H, dd, J = 12.3, 3.0 Hz, H-7). In addition, the presence of a signal at δ_C 190.7 (CH-17) in the attached proton test (APT) spectrum confirmed the presence of the aldehyde group. The position of the oxidation was defined at CH-17 as compound **7** corresponds with an overoxidation product of compound **6**, a behavior previously reported in allylic oxidation reactions.

Although the methyl group at C-16 in compound **1** is also allylic, compounds **6** and **7** correspond only with oxidation products from C-17, and two possible hypotheses are considered to explain this apparent regioselectivity. On the

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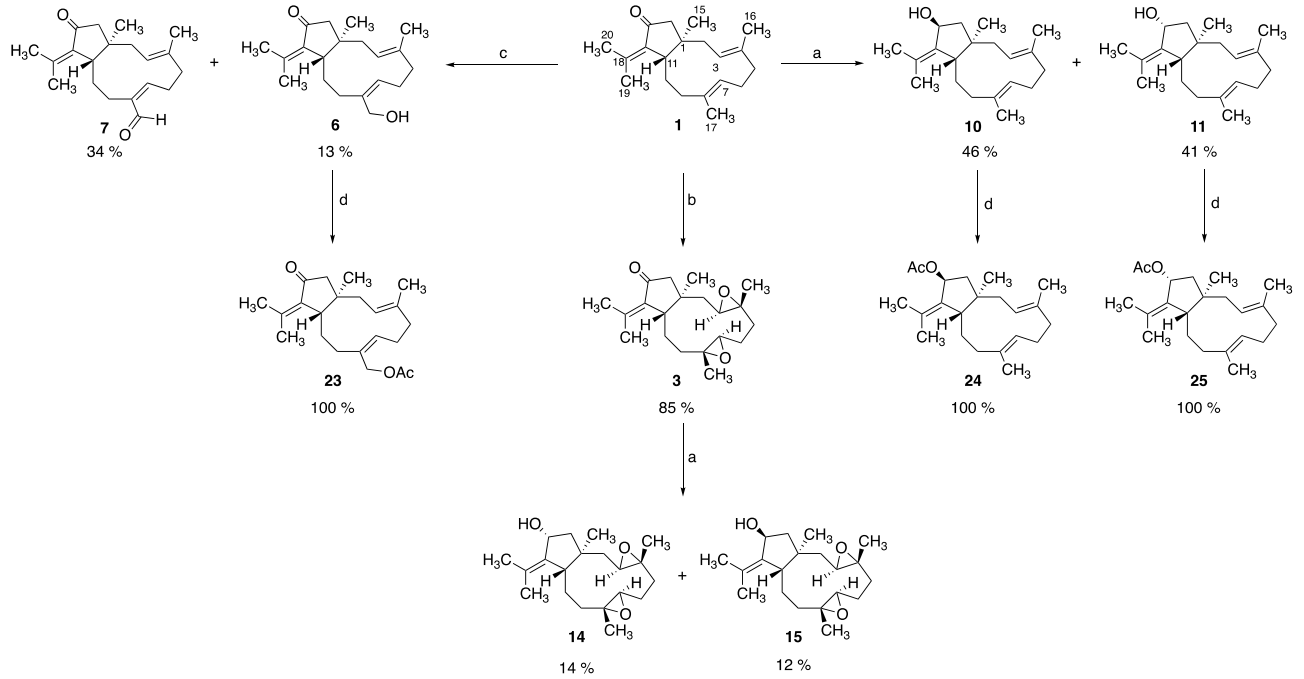
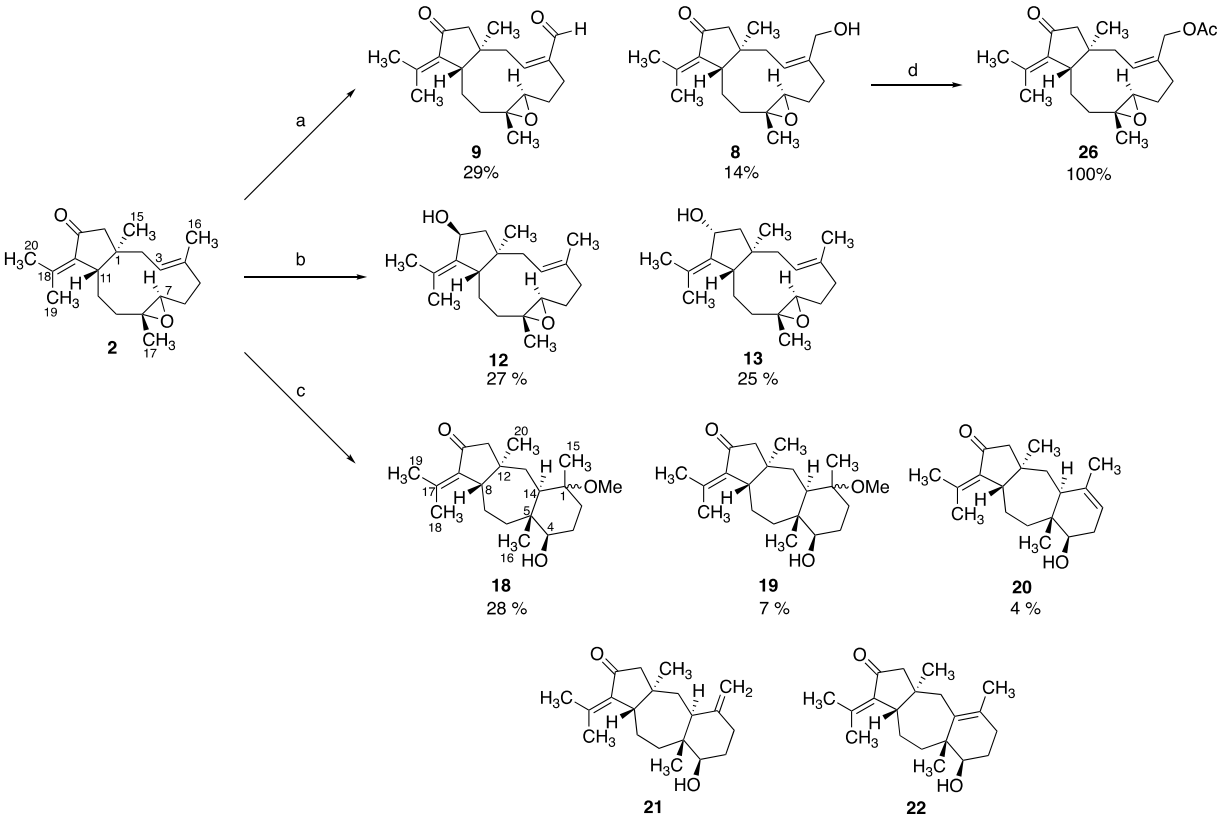
Scheme 1. Semisynthetic Derivatives Obtained from Dolabellatrienone (1)[a]



[a]Reaction conditions: (a) $NaBH_4$, MeOH; (b) m-CPBA, CH_2Cl_2; (c) SeO_2, ^tBuOOH, CH_2Cl_2; 18 h, rt; (d) Ac_2O, 4-DMAP, Et_3N.

Scheme 2. Semisynthetic Derivatives Obtained from Compound 2[a]



[a]Reaction conditions: (a) SeO_2, ^tBuOOH, CH_2Cl_2; (b) $NaBH_4$, MeOH; (c) p-TsOH, MeOH; (d) Ac_2O, DMAP, Et_3N.

one hand, a conformational analysis and optimization of geometry was conducted employing quantum mechanics calculations for compound **1** (see the Supporting Information for details). The optimized tridimensional structure of compound **1** shows that the methyl CH_3-16 is oriented toward the same face of the backbone of the dolabellane core, whereas the methyl CH_3-17 seems to be less sterically hindered. On the other hand, it is also possible that the

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remaining allylic oxidation products could be part of the high-polarity mixture detected by TLC. Further efforts to separate this mixture were not successful.

An allylic oxidation of compound **2** yielded compounds **8** and **9** (Scheme 2), whose structure elucidation process was conducted in the same way as for **6** and **7**. It is important to note that, compared to dolabellatrienone (**1**), only the methyl group at CH_3-16 in compound **2** is active toward allylic oxidation with SeO_2. The methyl at CH_3-17 is not active due to the presence of the epoxide group. Compound **8** exhibits signals in the ^{1}H NMR spectrum at δ_H 4.15 (1H, d, J = 11.6 Hz, H-16a) and δ_H 3.91 (1H, d, J = 11.6 Hz, H-16b) that correlate with a carbon in δ_C 58.5 (CH_2-16). These signals confirm the presence of an oxygenated methylene in compound **8**. The ^{1}H NMR spectrum of compound **9** shows signals at δ_H 9.72 (1H, d, J = 0.7 Hz, H-16/δ_C 189.9, CH-16) and δ_H 6.69 (1H, dd, J = 12.6, 5.9 Hz, H-3/δ_C 128.3, CH-3), consistent with the presence of an α,β unsaturated aldehyde. The elucidation of compounds **8** and **9** was completed by further analysis of correlations by 2D NMR spectroscopy experiments.

Reduction Reactions. Previous studies indicate that the presence of hydroxy groups is associated with an improvement of the antiviral activity in dolabellanes.[15] In this sense, the ketone carbonyl present in C-13 was a direct target with the aim to obtain hydroxylated derivatives on that position of the dolabellane core. Compounds **1**, **2**, and **3** were treated with sodium borohydride to obtain dolabellanes with primary and secondary alcohols (**10**−**15**).

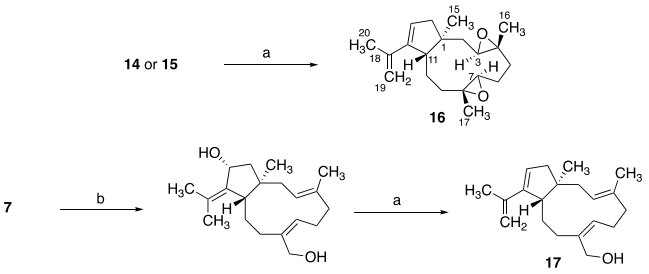
The reduction reaction of dolabellatrienone (**1**) with $NaBH_4$/EtOH afforded the epimeric alcohols **10** and **11** (Scheme 1). The ^{1}H NMR spectra for both compounds exhibit signals at δ_H 4.6, whereas APT spectra show signals at δ_C 72.0. These observations are consistent with the reduction of the ketone carbonyl at C-13. The spectroscopic properties of compound **10** are consistent with those reported for 13*S*-*epi*-isopalominol, and the reported ones for isopalominol are consistent with the spectroscopic data of compound **11**.[17]

Compound **2** was reduced under the same conditions to obtain compounds **12** and **13** (Scheme 2). For compound **12**, the ^{1}H and ^{13}C NMR spectra include a new signal at δ_H 4.67 (1H, d, J = 7.0 Hz, H-13/71.5, CH-13), whereas compound **13** exhibits a signal at δ_H 4.66 (1H, appt, J = 6.5 Hz, H-13/δ_C 71.6, CH-13). In both cases, the observed signals support the presence of an oxygenated methine. Both compounds were proposed as epimeric alcohols at C-13, and their absolute configurations were assigned based on the multiplicity exhibited by the H-13 proton. It is reported that oxygenated protons in dolabellanes with the 13*R* configuration appear as a triplet with a coupling constant of ∼6.5 Hz, whereas a 13*S* configuration is assigned when the signal appears as a doublet with a coupling constant at ∼7.0 Hz.[14,18] Our results were consistent with these observations, and this allowed us to propose that compound **12** corresponds with the 13*S* epimer whereas compound **13** is the 13*R* epimer.

In this work, the reduction of dolabellatrienone (**1**) and compound **2** yields the epimeric alcohols in equal quantities without any selectivity, a feature that is expected when a small reducing agent such as sodium borohydride is employed. Interestingly, previous work showed that a bulky reducing agent such as RED-Al did not improve the selectivity but decreased the reaction yield.[15] Surprisingly, in this work the reduction of compounds **1** and **2** under the Luche conditions ($NaBH_4$/$CeCl_3$) results in a different distribution of the epimeric alcohols, and selectivity toward the 13*S* epimer is observed. Compounds **10** and **11** are obtained in an 8:1 ratio, whereas compounds **12** and **13** are obtained in a 5:1 ratio.

The reduction of compound **3** afforded the compounds **14** and **15** (Scheme 1). Compound **14** exhibits a signal at δ_H 4.63 (1H, apparent t, J = 7.2 Hz, H-13) assigned to the new alcohol group, and the configuration was proposed as the 13*R* epimer based on the multiplicity and coupling constant value. Similarly, compound **15** was proposed as the 13*S* epimer due to the signal at δ_H 4.65 (1H, d, J = 6.7 Hz, H-13). The reaction yield of the reduction of compound **3** was disappointing compared with those obtained for the reduction of dolabellatrienone (**1**) and **2**. We observed that the reaction works without issues, but the obtained products show an unexpected lack of stability during the purification process. After unsuccessful efforts at purifying compounds **14** and **15** employing normal-phase column chromatography, a purification by reverse-phase (RP) high-performance liquid chromatography (HPLC) proved to separate these compounds. However, less than 10% of the separated material was recovered suggesting an unexpected lability of **14** and **15**. Furthermore, during the recording of two-dimensional NMR spectra, compounds **14** and **15** afford the same compound **16** by a dehydration process (Scheme 3). The NMR spectra of

Scheme 3. Dehydration of Compounds 7, 14, and 15[a]



[a]Conditions: (a) $CDCl_3$, rt; (b) $NaBH_4$, MeOH

compound **16** exhibited signals at δ_H 5.65 (1H, t, J = 2.4 Hz, H-13/δ_C 126.1, CH-13), δ_H 4.95 (1H, s, H-20a/δ_C 111.9, CH_2-20), and δ_H 4.90 (1H, s, H-20b/δ_C 111.9, CH_2-20), indicating the presence of two double bonds, one of them trisubstituted and the other 1,1-disubstituted. The correlations found in the heteronuclear single-quantum coherence (HSQC) and HMBC spectra allowed us to elucidate the complete structure of **16**. The transformation of cembrane diterpenes due to acid traces present in the deuterated chloroform has been reported previously.[19] Therefore, it is proposed that acid traces are responsible for the acid-catalyzed dehydration of compounds **14** and **15** to afford the conjugated diene **16**.

The presence of two carbonyl groups in compound **9** represents a valuable target to obtain hydroxylated compounds. Therefore, compound **9** was treated with $NaBH_4$/MeOH and a complex mixture of products was obtained. After a separation by column chromatography, one major compound could be isolated with signals in the ^{1}H NMR spectrum at δ_H 4.29 (1H, d, J = 11.2 Hz, H-17a) and 3.99 (1H, d, J = 11.2 Hz, H-17b) that indicate the presence of an oxygenated methylene, whereas a signal at δ_H 4.64 (1H, t, J = 6.6 Hz, H-13) suggested the appearance of an oxygenated methine. The

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observed signals allowed us to conclude that both carbonyls in compound **9** were reduced to obtain a dihydroxylated derivative with the 13*R* configuration (Figure S48). However, the same phenomenon of dehydration exhibited by compounds **14** and **15** was observed to yield compound **17** (Scheme 3). Compound **17** exhibited ^{1}H NMR signals at δ_H 5.59 (1H, s, H-13), 4.86 (1H, s, H-20a), and 4.63 (1H, s, H-20b) assigned to olefinic protons, two of them exomethylene protons. The structure of compound **17** was fully elucidated based on its 2D NMR data.

Epoxide Ring Opening. The epoxide moiety between C-7 and C-8 in compound **2** and compound **3** was used to generate hydroxylated derivatives through ring-opening reactions. First, these compounds were treated with *p*-toluensulfonic acid in tetrahydrofuran (THF)/H_2O mixtures. Under these conditions, compound **2** produced a complex mixture of products, but interestingly compound **3** did not show reaction products even after prolonged reaction times and heating. Therefore, compound **2** was treated with *p*-toluensulfonic acid in methanol to obtain compounds **18**−**22**.

Compound **18** shows a signal in its ^{1}H NMR spectrum for an oxygenated methine at δ_H 3.20 (1H, dd, J = 10.9, 3.2 Hz, H-4/δ_C 78.0, CH-4) together with an intense signal at δ_H 3.10 (3H, br d, J = 0.9 Hz, H-21/δ_C 47.7, CH_3-21). The latter signal suggested the presence of a methoxy group, and this was fully supported by the presence of 21 carbon atom signals in the APT spectrum. The observed signals reveal the successful opening of the epoxide ring, and compound **18** was elucidated by 2D NMR experiments. Key HMBC correlations between methyl protons at δ_H 0.99 (s, H_3-16/δ_C 78.0, CH_3-16) and carbons at δ_C 42.8 (C-5) and δ_C 49.0 (CH-14) suggested that the dolabellane core rearranged to produce a dolastane diterpene. The position of the methoxy group was deduced based on the HMBC correlation observed between its protons and the carbon at δ_C 76.1 (C-1). The remaining correlations allowed the deduction of the planar structure, and the configuration was proposed based on X-ray diffraction experiments. The compound crystallizes in the space group *R*3, with two independent molecules per asymmetric unit. Disorder is observed in a terminal group of one of the molecules, likely contributing to the inability to refine the structure's Flack parameter. Thus, the crystalline structure allowed us to establish the relative configuration of compound **18** (Figure S81 and Table S1).

Compound **19** shows ^{1}H NMR and APT spectra reminiscent of those of compound **18**. Signals in the ^{1}H NMR spectrum at δ_H 3.18 (1H, dd, J = 11.5, 4.2 Hz, H-4/δ_C 78.4, C-4) and δ_H 3.09 (s, H_3-21) evidence the presence of the oxygenated methine and the methoxy group. The only difference compared with compound **18** is observed for the methyl located on C-1. In compound **19**, this methyl group appears at δ_C 25.2 (C-15), whereas it is located at δ_C 19.1 (C-15) in compound **18**. Then, it is proposed that compounds **18** and **19** are epimers with an opposite configuration at C-1.

The ^{1}H NMR and APT spectra of compound **20** exhibited characteristic signals that were consistent with those of a dolastane diterpene. An olefinic proton at δ_H 5.35 (1H, br s, H-2) and an oxygenated methine at δ_H 3.48 (1H, dd, J = 10.3, 6.1 Hz, H-4) confirmed the opening of the epoxide. The identification of compound **20** was completed by a comparison with the spectroscopic properties reported for a dolastane diterpene with a double bond between C-1 and C-2.[20]

The remaining reaction product was inferred as a mixture of the compounds **21** and **22** based on a gas chromatography-mass spectrometry (GC-MS) analysis that revealed the mixture consisted of two isomeric compounds with m/z = 302. Moreover, this hypothesis was supported by the presence of 40 signals in the APT spectrum of the mixture. As compounds **21** and **22** could not be successfully separated by normal- and reversed-phase column chromatography, they were analyzed as the mixture. Signals at δ_H 4.83 (1H, d, J = 1.2 Hz, H-15a) and δ_H 4.63 (1H, d, J = 1.2 Hz, H-15b) together with carbons at δ_C 108.3 (C-15) and 44.8 (C-5) suggest the presence of a dolastane diterpene with an olefinic exomethylene. A detailed comparison with the spectroscopic properties reported for (4*R*,5*R*,8*S*,12*R*,14*S*)-10-keto-4-hydroxy-1(15),9(17)-dolastadiene (**21**) allowed us to conclude that it was present in the mixture.[20]

The remaining spectroscopic signals were used to elucidate the structure of compound **22** as follows. The APT spectrum of the mixture shows four signals at δ_C 146.1, 134.9, 133.4, and 129.2, all of them of quaternary double bonds, one signal at δ_C 72.2 for an oxygenated methine and five signals at 23.7, 23.3, 23.23, 21.1, and 19.1 assignable to methyl groups. The presence of a hydroxylated methine was confirmed by the signal in the ^{1}H NMR spectrum at δ_H 3.63 (1H, dd, J = 10.4, 4.2 Hz, H-4). The configuration of C-4 is proposed as 4*R* based on the coupling constant values of the H-4 proton, which are comparable to those in compounds **18**, **19**, **20**, and **21**. Considering that there are no remaining signals for protons in double bonds, **22** should correspond with a dolastane diterpene with a double bond between C-1 and C-14 and is proposed as (4*R*,5*R*,8*S*,12*R*,14*S*)-4-hydroxy-10-keto-1(14),9-(17)-dolastadiene.

Acetylation Reactions. Finally, with the aim to expand the diversity of our collection of compounds, the hydroxy-bearing compounds available in enough quantities were acetylated employing acetic anhydride (Ac_2O) and catalytic quantities of 4-dimethylaminopyridine (4-DMAP) (Scheme 2 and Scheme 3).

Compounds **6**, **8**, **10**, and **11** yielded the acetylated derivatives **23**−**26**. In all cases, the acetylation was judged by the appearance of NMR signals for a further methyl group and a carbonyl carbon from the acetate group. For instance, the ^{1}H NMR spectrum for compound **23** showed signals at δ_H 2.03 (3H, s, H-22), and the APT spectrum showed two further signals at δ_C 171.2 (C-21) and 21.5 (C-22). Similar signals were found in the NMR spectra of compounds **24**−**26**, confirming the presence of the acetyl group in these compounds.

Biological Activity. The biological activity assays were conducted for compounds with enough purity according to their ^{1}H and ^{13}C NMR spectra. First, the cytotoxicity in Vero cells was tested and expressed as the concentration that reduced cell viability by 50% when compared to untreated controls (CC_{50}). As Table 1 shows, the evaluated compounds showed no cytotoxicity ($CC_{50} \geq 100$ μM). The antiviral activities of the dolabellanes against ZIKV and CHIKV also were determined by adding the compounds, at a concentration of 20 μM, after the adsorption of the virus. On the one hand, the results in Table 1 show that compounds **9**, **12**, **15**, **18**, and **20** inhibit ZIKV virus replication in the range of 80−100% with specific values of 99 ± 1, 99 ± 3, 81 ± 4, 100, and 99 ± 1%, respectively. On the other hand, compounds **2**, **4**, **8**, **15**, and **25** were able to inhibit CHIKV replication with values of

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Table 1. Cytotoxicity and anti-ZIKAV and CHIKV Activities of Dolabellane and Dolastane Diterpenes

compound	CC_{50} (μM)	inhibition of viral replication (%)	
		ZIKV (at 20 μM)	CHIKV (at 20 μM)
dolabelladienetriol	400	50 ± 1	35 ± 2
1	150	0	0
2	530	38 ± 2	99 ± 1
3	960	56 ± 2	0
4	470	65 ± 3	82 ± 2
7	100	0	29 ± 4
8	800	60 ± 1	71 ± 3
9	750	99 ± 1	39 ± 4
10	190	51 ± 1	0
12	580	99 ± 3	45 ± 4
13	430	30 ± 3	33 ± 4
14	960	49 ± 3	44 ± 3
15	1000	81 ± 4	98 ± 2
16	480	60 ± 3	0
17	600	61 ± 4	0
18	730	100	25 ± 2
19	650	59 ± 2	40 ± 2
20	580	99 ± 1	65 ± 2
23	250	30 ± 4	0
25	100	62 ± 3	99 ± 1
ribavirin	300	93 ± 3	97 ± 1

99 ± 1, 82 ± 2, 71 ± 3, 98 ± 2, and 99 ± 1%, respectively. Ribavirin was used as a control at a concentration of 20 μM resulting in the inhibition of 93% and 97% replication of ZIKV and CHIKV, respectively. Interestingly, among the derivatives obtained from the reduction reactions, the 13*S* epimers (compounds **12** and **15**) are more active than the 13*R* epimers (**13** and **14**). Against both viruses, compounds **12** and **15** exhibit higher inhibition and less cytotoxicity compared with their epimers. Compounds **18** and **20**, representing dolastane diterpenes, exhibit remarkable ZIKV inhibition. In general, compounds **18**−**20** showed higher ZIKV inhibition compared with CHIKV, suggesting that ZIKV could represent a better molecular target for dolastane diterpenes.

The EC_{50} value, which is defined as the compound concentration that is required to inhibit viral replication by 50%, was determined using linear regression. For this analysis, ZIKV- and CHIKV-infected Vero cells were incubated with different concentrations of the compounds, starting from a concentration with an inhibitory potential of 50 μg/mL and declining progressively. At 72 h postinfection, the cells were lysed, the supernatants were harvested, and the virus yields were quantified by a plaque-reduction assay. Compounds **9**, **12**, **15**, **18**, and **20** tested for ZIKV exhibited high antiviral activity in a dose-dependent manner (Table 2), and compound **9** showed the lowest EC_{50} (0.90 ± 0.08 μM). For CHIKV, compounds **2**, **4**, **8**, **15**, and **25** were tested. Compounds **15** and **2** were the most active against CHIKV, with EC_{50} values equal to 0.70 ± 0.03 and 1.2 ± 0.1 μM, respectively. Because the selective index represents the degree of safety when a compound is used, it was calculated ($SI = CC_{50}/EC_{50}$) for each compound. Our results reinforce the promising profile of compound **9** (ZIKV), which exhibits an SI value of 830.

To evaluate the direct effect of compounds on ZIKV and CHIKV particles, a virucidal assay was performed. Using ZIKV, we observed 90% and 80% reductions in the virus titer after a treatment with 20 μM of compounds **9** and **12**, respectively. When the effect was evaluated using CHIKV, a significant virucidal effect was observed with compounds **2** and **25** (Figure 1).

To evaluate if the compounds inhibit the early stages of virus replication, we studied whether compounds **9**, **12**, **18**, **15**, and **20** at concentrations of 5, 10, and 20 μM could interfere with ZIKV adsorption and whether compounds **2**, **4**, **8**, **15**, and **25** at concentrations of 5, 10, and 20 μM could interfere with CHIKV adsorption (Figure 2A,B). Our results showed that compound **18** inhibited ZIKV adsorption to more than 90% at a concentration of 20 μM, whereas compounds **2** and **25** reduced CHIKV adsorption more than 90% at 20 μM.

Several studies have shown that molecules can interfere with a viral adsorption, with the mechanism of blocking viral target receptors.[21] The process of adsorption of arboviruses on the cell surface depends on the interaction between viral glycoproteins (especially E2) and cellular receptor(s). Our studies demonstrate that compounds **18** for ZIKV and **2** and **25** for Chikungunya appear to affect early events during a virus infection. This demonstrates that these compounds may be better studied as a strategy for the early treatment of arbovirus infections.

EXPERIMENTAL SECTION

General Experimental Procedure. Optical rotations were measured on a ADP440+ Polarimeter. Nuclear magnetic resonance experiments were conducted with Bruker AVANCE 400 and Varian 500 spectrometers in deuterated chloroform ($CDCl_3$ δ_H 7.26, δ_C 77.0) as the solvent. Electronic impact mass spectrometry (EIMS) spectra were obtained from an Agilent 5977A MSD mass spectrometer employing an electron impact at 70 eV in a range of m/z from 40 to 800. High-resolution mass spectrometry (HRMS) spectra were obtained in an Agilent 6545 LC/Q-TOF mass spectrometer. High-performance liquid chromatography separations were conducted with a Merck Hitachi equipment equipped with an L-6000A pump and an L-4250 UV/vis detector. A column LiChrospher

Table 2. Cytotoxicity (CC_{50}), anti-ZIKV or anti-CHIKV Profile (EC_{50}), and Selectivity Index (SI) of Selected Compounds[a]

ZIKV				CHIKV			
compounds	CC_{50},[b] (μM)	EC_{50},[c] (μM)	SI[d]	compounds	CC_{50},[b] (μM)	EC_{50},[c] (μM)	SI[d]
9	750	0.90 ± 0.08	830	**2**	530	1.2 ± 0.1	440
12	580	1.2 ± 0.1	480	**4**	470	9.5 ± 0.2	50
15	1000	8.9 ± 0.1	110	**8**	800	13 ± 1	61
18	730	1.8 ± 0.1	410	**15**	1000	0.70 ± 0.03	1400
20	580	2.1 ± 0.1	280	**25**	100	1.2 ± 0.1	83

[a]The mean values ± standard deviations are representative of three independent experiments. [b]Concentration that reduced 50% cytotoxic concentration when compared to untreated controls. [c]Concentration that reduced 50% of ZIKV or CHIKV replication when compared to infected controls. [d]Selectivity index was defined as the ratio between CC_{50} and EC_{50} and represents the safety for in vitro assays.

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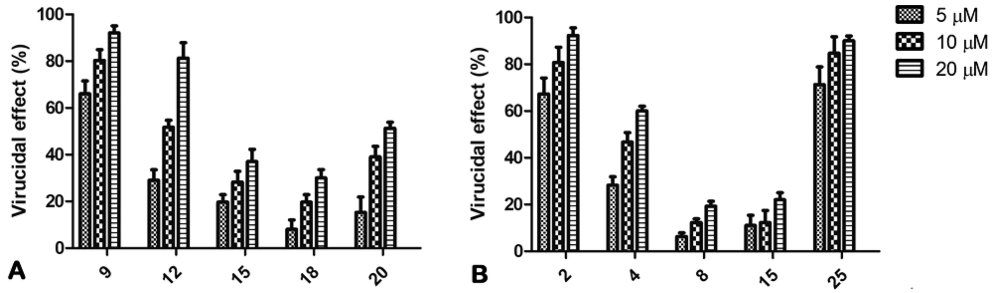

Figure 1. Effect of diterpenes on the infectivity of ZIKV or CHIKV. (A) Effect on the infectivity of ZIKV. (B) Effect on the infectivity of CHIKV. The viral suspensions (ZIKV and CHIKV) were incubated in the presence or absence of 5, 10, and 20 μM of the compounds and at 37 °C for 4 h. The results were evaluated by a plaque assay. Error bars indicate the standard deviation, and experiments were performed in triplicate.

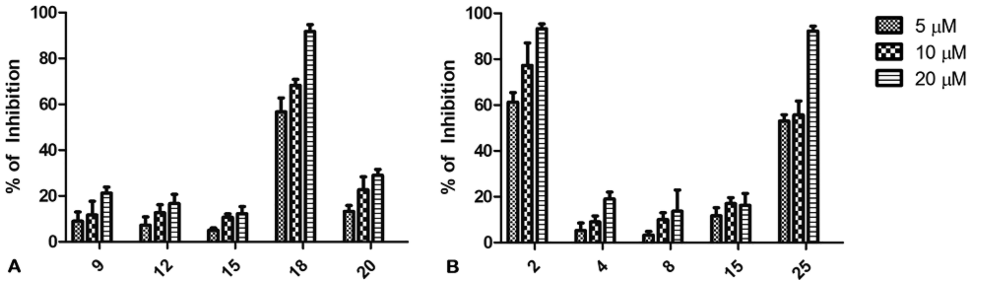

Figure 2. Effect of selected diterpenes on the adsorption of ZIKV or CHIKV. (A) Effect on the the adsorption of ZIKV. (B) Effect on the adsorption of CHIKV. Vero cells were infected with ZIKV or CHIKV on MOI of 0.1; the results were evaluated by a plaque assay. Error bars indicate the standard deviation, and experiments were performed in triplicate.

RP-18 (250 × 10 mm, i.d., 10 μm) and HPLC-grade methanol were utilized. Column chromatography separations were made with silica gel (0.043−0.060 mm, Merck), eluting with analytical-grade solvents acquired from Merck. Preparative TLC was developed on 20 × 20 cm glass plates with silica gel 60F_{254} (250 μm) as the stationary phase. The single-crystal X-ray diffraction data for compound **18** were obtained on a Bruker D8 Venture diffractometer using Cu Kα radiation (1.5418 Å). The data collection and cell refinement were performed using APEX (Bruker 2012: Bruker, 2012 APEX-III. Bruker AXS Inc.). All structures were solved using the direct method, and the refinement was performed by the full-matrix least-squares method in the SHELX program package. Non-hydrogen atoms were refined with anisotropic displacement parameters, and the hydrogen atoms were positioned geometrically using the riding model. The semisynthetic modifications were conducted under argon atmosphere, and solvents used were distilled before being used. All reactions were monitored by thin-layer chromatography with Merck aluminum plates precoated with silica gel 60F_{254}. The plates were visualized under UV light (λ = 254 nm) and posteriorly treated with a 5% cerium ammonium sulfate and 10% sulfuric acid solution in MeOH followed by heating. Sodium borohydride, trimethylamine, acetic anhydride, 4-dimethylaminopyridine 99%, and *p*-toluensulfonic acid were acquired from Merck. Anhydrous cerium chloride 99.5%, selenium oxide 99.4%, *tert*-butyl hydroperoxide 70% aqueous solution, and *m*-CPBA 70% were acquired from Alfa Aesar.

Animal Material. The soft corals *Eunicea laciniata* and *Eunicea asperula* were collected in Santa Marta, Colombian Caribbean Sea, by scuba diving in December 2011. Samples were frozen after the collection and remained in that condition until their extraction. M. Puyana identified the organisms, and vouchers were deposited at the collection of Instituto de Ciencias Naturales, Universidad Nacional de Colombia. *E. laciniata* was codified as ICN-MHN-CR 106, and *E. asperula* was coded with PO0267. The Ministerio de Ambiente, Vivienda y Desarrollo territorial granted permissions for the collection (Contracto de acceso a recurso genético No. 109). Compounds **1**−**5** were obtained by an extraction with CH_2Cl_2 and fractionation by column chromatography as reported.[16]

*Formation of Compound **3** by Epoxidation of Dolabellatrienone (**1**).* A solution of dolabellatrienone (**1**) (44.8 mg, 0.157 mmol) and m-CPBA (87.5 mg, 0.356 mmol) in CH_2Cl_2 (5.0 mL) was stirred at room temperature (rt) for 4 h. The organic phase was washed with saturated solutions of Na_2SO_3, $NaHCO_3$, and NaCl (2 × 3.0 mL) and dried with sodium sulfate. Then, the organic phase was concentrated, and the residue was purified by silica gel column chromatography (*n*-hexane/ethyl acetate (EtOAc) 6:4) to obtain compound **3** (42.2 mg, 84.7%).

*(1R,3R,4R,7R,8R,11S)-3,4:7,8-Diepoxy-13-ketodolabell-12(18)-ene (**3**).* White solid; $[\alpha]_D^{21}$ −21 (*c* 1.14, $CHCl_3$); ^{1}H and ^{13}C NMR are consistent with those reported by Wei et al.[22] EIMS *m*/*z* 318 $[M]^+$ (48), 275 $[M - C_3H_7]^+$ (7), 233 (1), 217 (10), 203 (8), 189 (13), 175 (14), 161 (22), 149 (100), 135 (73), 121 (62), 109 (69), 93 (76), 79 (63), 67 (51), 55 (72).

*Formation of Compounds **6** and **7** by an Allylic Oxidation of Dolabellatrienone (**1**).* A solution of SeO_2 (5.1 mg, 0.050 mmol) and *t*-butyl hydroperoxide (50.0 μL, 0.363 mmol) in CH_2Cl_2 (1.0 mL) was stirred at rt for 30 min. Then, dolabellatrienone (**1**) (68.3 mg, 0.239 mmol) dissolved in CH_2Cl_2 (4.0 mL) was added, and the resulting solution was stirred at rt for 3 h. The mixture was then partitioned with H_2O (5.0 mL), and the organic phase was treated with $NaHCO_3$ and NaCl (2 × 3.0 mL) saturated solutions. The organic phase was dried with sodium sulfate and concentrated under vacuum. The obtained residue (74.5 mg) was purified by column chromatography (*n*-hexane/EtOAc 9:1−6:4) to obtain compound **6** (9.2 mg, 13.0%) and compound **7** (24.3 mg, 34.0%).

*(1R,3E,7Z,11S)-17-Hydroxy-13-ketodolabella-3,7,12(18)-triene (**6**).* Colorless oil; $[\alpha]_D^{23}$ +6.6 (*c* 0.04, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 300 MHz) δ_H 5.27 (1H, dd, *J* = 11.2, 5.1 Hz, H-3), 5.19 (1H, m, H-7), 4.29 (1H, d, *J* = 11.2 Hz, H17a), 3.98 (1H, d, *J* = 11.2 Hz, H-17b), 2.74 (1H, br d *J* = 11.5 Hz, H-11), 2.46 (1H, m, H-6a), 2.43 (1H, m, H-9a), 2.38 (1H, br d, *J* = 18.3 Hz, H-14a), 2.28 (1H, m, H-5a), 2.26 (1H, m, H-9b), 2.22 (1H, m, H-6b), 2.21 (3H, s, H-20), 2.19 (1H, m, H-5b), 2.10 (1H, br d *J* = 18.3 Hz, H-14b), 2.10 (1H, m, H-2a), 1.74 (3H, s, H-19), 1.67 (1H, m, H-10a), 1.60 (1H, m, H-2b), 1.42 (3H, s, H-16), 1.39 (1H, m, H-10b), 1.22 (3H, s, H-15); ^{13}C NMR ($CDCl_3$, 75 MHz) δ_C 207.1 (C, C-13), 148.4 (C, C-18), 137.8 (C, C-12), 136.3 (C, C-8), 135.5 (C, C-4), 135.0 (CH, C-7), 125.2 (CH, C-3),

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59.7 (CH_2, C-17), 54.8 (CH_2, C-14), 41.6 (CH, C-11), 41.0 (C, C-1), 40.0 (CH_2, C-2), 39.6 (CH_2, C-5), 34.5 (CH_2, C-9), 28.3 (CH_2,C-10), 24.5 (CH_3, C-19), 23.8 (CH_2, C-6), 23.1 (CH_3, C-15), 21.3 (CH_3, C-20), 15.6 (CH_3, C-16); EIMS m/z 302 $[M]^+$ (2), 269 $[M - CH_3 - H_2O]^+$ (6), 241 $[M - C_3H_7 - H_2O]^+$ (4), 215 (3), 187 (6), 175 (12), 163 (16), 150 (98), 135 (58), 121 (35), 107 (56), 91 (100), 79 (94), 67 (80), 55 (79); HR electrospray ionization (ESI) MS m/z 303.2322 $[M + H]^+$ (calcd for $C_{20}H_{31}O_2$, 303.2324).

*(1R,3E,7Z,11S)-13-Ketodolabella-3,7,12(18)-trien-17-al (**7**).* White solid; $[\alpha]_D^{23}$ +60 (c 0.1, $CHCl_3$); ^1H NMR ($CDCl_3$, 300 MHz) δ_H 10.09 (1H, s, H-17), 6.40 (dd, J = 12.3, 3.0 Hz, 1H, H-7), 5.39 (1H, dd, J = 11.3, 5.1 Hz, H-3), 2.78 (1H, br d, J = 10.1 Hz, H-11), 2.38 (1H, d, J = 18.2 Hz, H-14a), 2.18 (3H, s, H-20), 2.09 (1H, d, J = 18.2 Hz, H-14b), 1.59 (3H, s, H-19), 1.46 (3H, s, H-16), 1.22 (3H s, H-15); ^{13}C NMR (75 MHz, $CDCl_3$) δ_C 206.6 (C, C-13), 190.7 (CH, C-17), 154.4 (CH, C-7), 149.5(C, C-18), 138.3 (C, C-12), 137.0 (C, C-8), 135.3 (C, C-4), 125.8 (CH, C-3), 54.7 (CH_2, C-14), 41.7 (CH, C-11), 40.8 (C, C-1), 39.8 (CH_2, C-2), 39.4 (CH_2,C-5), 30.7 (CH_2,C-9), 28.0 (CH_2, C-10), 25.0 (CH_3, C-15), 23.3 (CH_2, C-6), 22.8 (CH_3, C-19), 21.3 (CH_3, C-20), 15.5 (CH_3, C-16); EIMS m/z 300 $[M]^+$ (14), 272 (4), 257 $[M- C_3H_7]^+$ (6), 239 $[M- C_3H_7- H_2O]^+$ (4), 219 (2), 203 (5), 189 (8), 176 (11), 161 (10), 150 (100), 135 (51), 121 (18), 107 (34), 91 (46), 67 (31), 55 (21); HRESIMS m/z 301.2089 $[M + H]^+$ (calcd for $C_{20}H_{29}O_2$, 301.2168).

*Formation of Compounds **8** and **9** by an Allylic Oxidation of Compound **2**.* A solution of SeO_2 (6.3 mg, 0.057 mmol) and t-butyl hydroperoxide (60.0 μL, 0.436 mmol) in CH_2Cl_2 (1.0 mL) was stirred at rt for 30 min. Afterward, compound **2** (73.2 mg, 0.242 mmol) dissolved in CH_2Cl_2 (4.0 mL) was added, and the resulting solution was stirred for 18 h at rt. Then, the mixture was partitioned with H_2O (5.0 mL), and the organic phase was washed with saturated $NaHCO_3$ and NaCl (2 × 3.0 mL) solutions, dried with sodium sulfate, and concentrated. The obtained residue (74.5 mg) was purified by silica gel column chromatography (n-hexane/EtOAc 9:1−7:3) to obtain compound **9** (22.5 mg, 29.3%) and compound **8** (10.6 mg, 13.7%). Compound **2** was also recovered (14.6 mg), and yields were calculated with respect to the quantity of **2** that reacted.

*(1R,3Z,7R,8R,11S)-7,8-Epoxy-16-hydroxy-13-ketodolabell-3,12(18)-diene (**8**).* Colorless oil; $[\alpha]_D^{23}$ + 63 (c 0.06, $CHCl_3$); ^1H NMR ($CDCl_3$, 400 MHz) δ_H 5.58 (1H, dd, J = 11.9, 5.1 Hz, H-3), 4.15 (1H, d, J = 11.6 Hz, 16a), 3.91 (1H, d, J = 11.6 Hz, H-16b), 2.87 (1H, d, J = 8.8 Hz, H-7), 2.76 (1H, d, J = 12.9 Hz, H-5a), 2.52 (1H, d, J = 12.5 Hz, H-11), 2.41 (1H, d, J = 18.5 Hz, H-14a), 2.26 (3H, s, H-20), 2.26 (1H, m, H-5b), 2.23 (1H, m, H-2a), 2.20 (d, J = 12.2 Hz, 1H), 2.14 (1H, d, J = 18.5 Hz, H-14b), 2.04 (1H, m, H-9a), 1.94 (3H, s, H-19), 1.92 (1H, m, H-6a), 1.78 (1H, m, H-6b), 1.72 (1H, m, H-2b), 1.63 (1H, m, H-10a), 1.51 (1H, m, H-10b), 1.37 (1H, m, H-9b), 1.34 (3H, s, H-17), 1.20 (3H, s, H-15); ^{13}C NMR ($CDCl_3$, 100 MHz) δ_C 206.4 (C, C-13), 149.8 (C, C-18), 128.3 (CH, C-3), 139.4 (C, C-4), 137.2 (C, C-12), 65.5 (CH, C-7), 60.5 (C, C-8), 58.5 (CH_2, C-16), 54.4 (CH_2, C-14), 42.0 (CH, C-11), 40.5 (C, C-1), 39.6 (CH_2, C-2), 36.8 (CH_2, C-9), 33.0 (CH_2, C-5), 27.3 (CH_2, C-10), 25.0 (CH_3, C-19), 23.1 (CH_2, C-6), 23.4 (CH_3, C-15), 21.9 (CH_3, C-20), 17.6 (CH_3, C-17). EIMS m/z 318 $[M]^+$ (4), 285 $[M- CH_3- H_2O]^+$ (4), 257 $[M- C_3H_7- H_2O]^+$ (3), 229 (3), 207 (12), 189 (21), 163 (37), 150 (95), 136 (90), 121 (64), 107 (70), 91 (100), 79 (91), 67 (58), 55 (75). HRESIMS m/z 319.2268 $[M + H]^+$ (calcd for $C_{20}H_{29}O_2$, 319.2273).

*(1R,3Z,7R,8R,11S)-7,8-Epoxy-13-ketodolabell-3-en-16-al (**9**).* White solid; $[\alpha]_D^{23}$ +70 (c 0.05, $CHCl_3$); ^1H NMR ($CDCl_3$, 400 MHz) δ_H 9.72 (1H, d, J = 0.7 Hz, H-16), 6.69 (1H, dd, J = 12.6, 5.9 Hz, H-3), 2.93 (1H, m, H-5a), 2.89 (1H, t, J = 12.5 Hz, H-7), 2.88 (1H, m, H-2a), 2.68 (1H, m, H-11), 2.51 (1H, br d, J = 18.5 Hz, H-14a), 2.25 (1H, br d, J = 18.5 Hz, H-14b), 2.24 (3H, s, H-19), 2.06 (1H, m, H-5b), 2.04 (1H, m, H-9a), 1.97 (1H, m, H-2b), 1.95 (1H, m, H-6a), 1.84 (3H, s, H-20), 1.81 (1H, m, H-6b), 1.68 (1H, m, H-10a), 1.49 (1H, m, H-10b), 1.30 (1H, m, H-9b), 1.28 (3H, s, H-17), 1.25 (3H, s, H-15); ^{13}C NMR ($CDCl_3$, 100 MHz) δ_C 205.0 (C, C-13), 189.9 (CH, C-16), 150.8 (C, C-18), 147.9 (CH, C-3), 142.0 (C, C-4), 136.3 (C, C-12), 65.3 (CH, C-7), 60.4 (C, C-8), 54.6 (CH_2, C-14), 41.6 (CH, C-11), 40.5 (C, C-1), 38.5 (CH_2, C-2), 36.6 (CH_2, C-9), 30.3 (CH_2, C-5), 27.1 (CH_2, C-10), 25.0 (CH_3, C-19), 23.4 (CH_2, C-6), 23.2 (CH_3, C-15), 21.8 (CH_3, C-20), 17.4 (CH_3, C-17); EIMS m/z 316 $[M]^+$ (6), 273 $[M- C_3H_7]^+$ (2), 229 (2), 207 (9), 189 (15), 175 (15), 163 (34), 149 (100), 135 (85), 121 (43), 107 (50), 91 (74), 79 (68), 67 (41), 55 (55). HRESIMS m/z 317.2113 $[M + H]^+$ (calcd for $C_{20}H_{29}O_3$, 317.2117).

*Formation of Compounds **10** and **11** by a Reduction of Dolabellatrienone (**1**).* Method A: Sodium borohydride (21.6 mg, 0.571 mmol) was added to a solution of dolabelletrienone (**1**) (47.6 mg, 0.166 mmol) in EtOH (3.0 mL) with stirring at rt. After 3 h, a mixture of H_2O/acetone 1:1 was added (1.0 mL), and it was diluted with EtOAc (10.0 mL). The organic phase was treated with $NaHCO_3$- and NaCl-saturated solutions, dried with sodium sulfate, and concentrated. The obtained residue (53.3 mg) was purified by silica gel column chromatography eluting with n-hexane/EtOAc (95:5) to obtain compound **10** (15.5 mg, 46.4%), compound **11** (13.6 mg, 40.7%), and recovered dolabellatrienone (**1**) (14.4 mg). Yields were calculated based on the quantity of dolabellatrienone (**1**) that reacted.

Method B: A solution of dolabellatrienone (**1**) (50.0 mg, 0.175 mmol) was treated with sodium borohydride (10.6 mg, 0.280 mmol) and $CeCl_3$ (51.6 mg, 0.209 mmol) with stirring at rt for 1.5 h. Then, EtOAc was added (7.0 mL), and the organic phase was extracted with $NaHCO_3$- and NaCl-saturated solutions. After the organic phase was concentrated under vacuum, the residue was purified by silica gel column chromatography eluting with n-hexane/AcOEt 95:5 to obtain compound **10** (17.3 mg, 43.0%), compound **11** (2.1 mg, 5.2%), and dolabellatrienone (**1**) (10.1 mg). Yields were calculated based on the quantity of dolabellatrienone (**1**) that reacted.

*13S-epi-Isopalominol (**10**).* Colorless oil; $[\alpha]_D^{23}$ −35 (c 0.05, $CHCl_3$); ^1H and ^{13}C NMR were consistent with those reported by Rodriguez et al.;[17] EIMS m/z 288 $[M]^+$ (1), 270 $[M-H_2O]^+$ (10), 255 $[M- CH_3- H_2O]^+$ (9), 227 $[M- C_3H_7- H_2O]^+$ (10), 205 (7), 187 (11), 173 (8), 159 (21), 147 (22), 134 (77), 119 (72), 105 (69), 91 (81), 79 (71), 67 (100), 55 (83).

*Isopalominol (**11**).* White solid; $[\alpha]_D^{23}$ −14 (c 0.057, $CHCl_3$); ^1H and ^{13}C NMR spectra were consistent with those reported by Rodriguez et al.;[17] EIMS 288 $[M]^+$ (0.8), 270 $[M-H_2O]^+$ (5), 255 $[M-CH_3- H_2O]^+$ (6), 227 $[M- C_3H_7- H_2O]^+$ (4), 205 (5), 187 (14), 173 (8), 159 (16), 152 (25), 134 (60), 121 (77), 105 (70), 91 (76), 79 (66), 67 (100), 55 (75).

*Formation of Compounds **12** and **13** by a Reduction of Compound **2**.* Method A: A solution of compound **2** (29.4 mg, 0.0974 mmol) in MeOH (2.0 mL) was treated with $NaBH_4$ (6.1 mg, 0.161 mmol) with stirring at rt for 4 h. EtOAc (3.0 mL) was added, and the organic phase was washed with sodium sulfate and concentrated. The residue (32.3 mg) was purified by silica gel column chromatography eluting with n-hexane/AcOEt 9:1 to obtain compound **12** (8.2 mg, 27.0%) and compound **13** (7.4 mg, 25.0%).

Method B: $NaBH_4$ (24.1 mg, 0.638 mmol) and $CeCl_3$ (30.1 mg, 0.122 mmol) were added to a solution of compound **2** (29.3 mg, 0.0970 mmol) in MeOH (2.0 mL). The mixture was stirred at rt for 6 h, and it was diluted with H_2O (10.0 mL). Then, it was extracted with EtOAc (3 × 5.0 mL), and the organic phases were dried with sodium sulfate and concentrated. The colorless residue (30.6 mg) was purified by silica gel column chromatography eluting with n-hexane/AcOEt 9:1 to obtain compound **12** (4.0 mg, 13.6%) and compound **13** (19.7 mg, 66.8%).

*(1R,7R,8R,11S,13S)-7,8-Epoxy-13-hydroxy-dolabella-3,12(18)-diene (**12**).* White solid; $[\alpha]_D^{23}$ −15 (c 0.1, $CHCl_3$); ^1H NMR ($CDCl_3$, 400 MHz) δ_H 5.43 (1H, dd, J = 11.7, 4.6 Hz, H-3), 4.66 (1H, d, J = 7.0 Hz, H-13), 2.87 (1H, d, J = 8.9 Hz, H-7), 2.57 (1H, t, J = 12.3 Hz, H-2a), 2.47 (1H, m, H-11), 2.28 (2H, m, H-5), 1.96 (1H, m, H-9a), 1.93 (1H, m, H-14a), 1.85 (3H, s, H-19), 1.84 (1H, m, H-6a), 1.77 (3H, s, H-20), 1.69 (1H, m, H-6b), 1.67 (m, 1H, H-2b), 1.65 (1H, m, H-14b), 1.59 (3H, s, H-16), 1.44 (2H, m, H-10), 1.36 (3H, s, H-17), 1.32 (1H, m, H-9b), 1.06 (3H, s, H-15); ^{13}C NMR ($CDCl_3$, 100 MHz) δ_C 147.4 (C, C-12), 135.1 (C, C-4), 130.9 (C, C-18), 126.1 (CH, C-3), 71.5 (CH, C-13), 65.8 (CH, C-7), 60.8 (C, C-

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8), 49.7 (CH_2, C-14), 48.0 (C, C-1), 43.4 (CH, C-11), 39.9 (CH_2, C-2), 38.1 (CH_2, C-5), 36.8 (CH_2, C-9), 27.4 (CH_2, C-10), 23.5 (CH_3, C-15), 23.0 (CH_2, C-6), 22.5 (CH_3, C-20), 22.1 (CH_3, C-19), 18.0 (CH_3, C-17), 15.6 (CH_3, C-16); EIMS *m/z* 286 $[M- H_2O]^+$ (0.9), 271 $[M- CH_3- H_2O]^+$ (1), 253 (1), 217 (0.1), 189 (3), 173 (9), 159 (11), 147 (27), 133 (75), 119 (53), 105 (65), 91 (72), 79 (62), 67 (76), 55 (100).

*(1R,7R,8R,11S,13R)-7,8-Epoxy-13-hydroxy-dolabella-3,12(18)-diene (**13**).* Colorless oil; $[\alpha]_D^{23}$ −35 (*c* 0.1, $CHCl_3$); ^1H NMR ($CDCl_3$, 400 MHz) δ_H 5.38 (1H, dd, *J* = 11.7, 4.2 Hz, H-3), 4.66 (1H, t, *J* = 6.5 Hz, H-13), 2.85 (1H, d, *J* = 8.8 Hz, H-7), 1.83 (3H, s, H-19), 1.75 (3H, s, H-20), 1.55 (3H s, H-16), 1.35 (3H s, H-17), 1.14 (3H, s, H-15); ^{13}C NMR ($CDCl_3$, 100 MHz) δ_C 145.1 (C, C-12), 135.4 (C, C-4), 130.0 (C, C-18), 125.5 (CH, C-3), 71.6 (CH, C-13), 66.1 (C, C-8), 60.8 (CH, C-7), 51.2 (CH_2, C-14), 46.5 (C, C-1), 42.7 (CH_2, C-11), 40.4 (CH_2, C-2), 38.0 (CH_2, C-5), 37.1 (CH_2, C-9), 28.6 (CH_2, C-10), 24.0 (CH_3, C-15), 23.0 (CH_2, C-6), 22.0 (CH_3, C-19), 21.2 (CH_3, C-20), 17.5 (CH_3, C-17), 15.5 (CH_3, C-16); EIMS *m/z* 304 $[M]^+$ (0.3), 286 $[M- H_2O]^+$ (0.9), 261 $[M- C_3H_7]^+$ (2), 243 $[M- C_3H_7- H_2O]^+$ (1), 220 (3), 191 (7), 173 (10), 133 (74), 121 (63), 105 (68), 91 (70), 79 (61), 67 (75), 55 (100). HRESIMS *m/z* 305.2476 $[M + H]^+$ (calcd for $C_{20}H_{33}O_2$ 305.2481).

*Formation of Compounds **14** and **15** by a Reduction of Compound **3**.* Compound **3** (21.9 mg; 0.0689 mmol) was disolved in EtOH (1.5 mL), and $NaBH_4$ (8.3 mg, 0.220 mmol) was added with stirring at rt. After 2.5 h, TLC monitoring indicated compound **6** reacted completely, and H_2O:/acetone 1:1 was added. The mixture was diluted with CH_2Cl_2 (5.0 mL), and the organic phase was washed with $NaHCO_3$- and NaCl-saturated solutions. The organic phase was concentrated, and the residue (25.6 mg) was purified by RP-HPLC (acetonitrile (ACN)/H_2O 60:40 v/v) to obtain compound **14** (3.1 mg, 14%) and compound **15** (2.6 mg, 12%). During the NMR analysis (deuterated chloroform 99.8%), compounds **14** and **15** transformed into compound **16**.

*(1R,3R,4R,7R,8R,11S,13R)-3,4:7,8-Diepoxy-13-hydroxy-dolabell-12(18)-ene (**14**).* Colorless oil; ^1H NMR ($CDCl_3$, 400 MHz) δ_H 4.63 (1H, t, *J* = 7.2 Hz, H-13), 3.00 (1H, dd, *J* = 11.1, 2.8 Hz, H-3), 2.90 (1H, d, *J* = 7.8 Hz, H-7), 2.44 (1H, d, *J* = 12.6 Hz, H-11), 1.81 (3H, s, H-19), 1.77 (3H, s, H-20), 1.42 (3H, s, H-16), 1.27 (3H, s, H-17), 1.20 (3H, s, H-15). EIMS *m/z* 320 $[M]^+$ (5), 302 $[M- H_2O]^+$ (2), 207 (7), 173 (10), 149 (22), 133 (82), 121 (43), 109 (48), 98 (100), 91 (46), 83 (72), 67 (37), 55 (61).

*(1R,3R,4R,7R,8R,11S,13S)-3,4:7,8-Diepoxy-13-hydroxy-dolabell-12(18)-ene (**15**).* Colorless oil; ^1H NMR ($CDCl_3$, 400 MHz) δ_H 4.65 (1H, d, *J* = 6.7 Hz, H-13), 3.03 (1H, dd, *J* = 9.4, 4.8 Hz, H-3), 2.93 (1H, d, *J* = 7.9 Hz, H-7), 2.61 (1H, d, *J* = 11.1 Hz, H-11), 1.83 (3H, s, H-19), 1.79 (3H, s, H-20), 1.43 (3H, s, H-16), 1.25 (3H, s, H-17), 1.21 (3H, s, H-15). EIMS *m/z* 320 $[M]^+$ (5), 302 $[M- H_2O]^+$ (0.7), 277 $[M- C_3H_7]^+$ (0.1), 223 (0.6), 201 (3), 173 (12), 147 (21), 133 (81), 121 (38), 109 (51), 98 (100), 83 (75), 67 (40), 55 (65).

*(1R,3R,4R,7R,8R,11S)-3,4:7,8-Diepoxy-13-dolabell-12,18(20)-diene (**16**).* Colorless oil; ^1H NMR ($CDCl_3$, 500 MHz) δ_H 5.65 (1H, t, *J* = 2.4 Hz, H-13), 4.95 (1H, s, H-20a), 4.90 (1H, s, H-20b), 3.06 (1H, dd, *J* = 11.4, 2.8 Hz, H-3), 2.87 (1H, d, *J* = 8.1 Hz, H-7), 2.56 (1H, d, *J* = 12.0 Hz, H-11), 2.45 (1H, d, *J* = 17.5 Hz, H-14a), 2.26 (1H, m, H-5a), 2.09 (1H, dd, *J* = 17.5, 3.3 Hz, H-14b), 2.03 (1H, m, H-9a), 2.00 (1H, m, H-6a), 1.89 (3H, s, H-19), 1.75 (1H, m, H-6b), 1.73 (1H, m, H-2a), 1.72 (1H, m, H-10a), 1.54 (1H, m, H-2b), 1.51 (1H, s, H-10b), 1.54 (3H, s, H-2b), 1.43 (1H, s, H-9b), 1.38 (3H, s, H-17), 1.36 (1H, m, H-5b), 1.31 (3H, s, H-15), 1.24 (3H, s, H-16); ^{13}C NMR (125 MHz, $CDCl_3$) δ_c 148.4 (C, C-12), 139.8 (C, C-18), 126.1 (CH, C-13), 111.9 (CH_2, C-20), 64.4 (CH, C-7), 63.4 (CH, C-3), 61.1 (C, C-8), 61.0 (C, C-4), 49.0 (CH_2, C-14), 46.3 (CH, C-11), 43.9 (C, C-1), 40.6 (CH_2, C-2), 37.7 (CH_2, C-5), 36.3 (CH_2, C-9), 25.8 (CH_2, C-10), 23.7 (CH_2, C-6), 22.9 (CH_3, C-15), 22.2 (CH_3, C-19), 17.3 (CH_3, C-17), 16.4 (CH_3, C-16).

*Formation of Compound **17** from Compound **9**.* Compound **9** (26.5 mg, 0.088 mmol) was dissolved in EtOH (1.5 mL), and 10.6 mg (0.28 mmol) of $NaBH_4$ was added at rt with constant stirring. After 20 h, 1 mL of a H_2O/acetone (1:1) solution was added, and the solution was partitioned between H_2O (5 mL) and EtOAc (5 mL). The organic phase was treated with a saturated solution of $NaHCO_3$ (2 × 4.0 mL) and dried with sodium sulfate. The organic phase was concentrated to obtain a residue (28.8 mg) that was purified by silica gel column chromatography eluting with *n*-hexane/EtOAc 9:1 to 1:1, obtaining 4.9 mg (18.2%) of a major product with ^1H NMR ($CDCl_3$, 500 MHz) δ_H 5.22 (1H, dd, *J* = 11.5, 4.8 Hz, H-3), 5.13 (1H, m, H-7), 4.64 (1H, t, *J* = 6.6 Hz, H-13), 4.29 (1H, d, *J* = 11.2 Hz, H-17a), 3.99 (1H, d, *J* = 11.2 Hz, H-17b), 1.79 (3H, s, H-16), 1.59 (3H, s), 1.41 (3H, s), 1.18 (3H, s). During an NMR analysis (deuterated chloroform 99.8%), the major compound transformed into compound **17**.

*(1R,3E,7Z,11S,12Z)-17-Hydroxy-dolabella-3,7,12,18(20)-tetraene (**17**).* ^1H NMR ($CDCl_3$, 300 MHz) δ_H 5.59 (1H, s, H-13), 5.30 (1H, dd, *J* = 11.9, 4.5 Hz, H-3), 5.14 (1H, d, *J* = 9.5 Hz, H-7), 4.86 (1H, s, H-20a), 4.63 (1H, s, H-20b), 4.28 (1H, d, *J* = 11.2 Hz, H-17a), 3.97 (1H, d, *J* = 11.2 Hz, H-17b), 1.87 (3H, s, H-19), 1.45 (3H, s, H-16), 1.19 (3H, s, H-15); ^{13}C NMR ($CDCl_3$, 75 MHz) δ_C 148.7 (C-12), 140.2 (C-18), 136.9 (C-8), 135.0 (C-4), 134.1 (C-7), 126.1 (C-13), 125.4 (C-3), 111.3 (C-20), 59.7 (C-17), 48.6 (C-14), 47.5 (C-1), 45.4 (C-11), 40.6 (C-2), 40.1 (C-5), 34.5 (C-9), 26.3 (C-10), 24.1 (C-6), 22.8 (C-15), 21.9 (C-19), 16.2 (C-16).

*Formation of Compounds **18**–**22** by an Acid-Catalyzed Epoxide Opening of Compound **4**.* Compound **4** (55.0 mg, 0.182 mmol) dissolved in MeOH (1.5 mL) was treated with *p*-toluensulfonic acid (2.5 mg, 0.013 mmol) at rt. After 2 h, 10.0 mg of sodium bicarbonate was added, and stirring was maintained for 10 min. The mixture was concentrated, and the residue was dissolved in CH_2Cl_2. The organic phase was washed with a NaCl saturated solution and dried with sodium sulfate. The residue obtained after concentration was purified by silica gel column chromatography (*n*-hexane/EtOAc 9:1–7:3) to obtain compound **18** (17.3 mg, 28.4%), compound **19** (4.1 mg, 7.0%), and 10.8 mg of a mixture of compounds **20**–**22**. The mixture of compounds **20**–**22** was purified through preparative TLC (*n*-hexane/*tert*-butyl methyl ether (TBME) 1:1) to obtain pure compound **20** (1.9 mg, 3.5%) and a 4.2 mg of mixture of compounds **21** and **22** (ratio 1:1 determined by GCMS).

(1R,4R,5R,8S,12R,14R)-4-Hydroxy-10-keto-1-methoxydolast-9-(17)ene (**18**).* White solid; $[\alpha]_D$ + 20 (*c* 0.05, $CHCl_3$); ^1H NMR ($CDCl_3$, 400 MHz) δ_H 3.20 (1H, dd, *J* = 10.9, 3.2 Hz, H-4), 3.10 (3H, br d, *J* = 0.9 Hz, H-21), 2.88 (1H, m, H-8), 2.18 (3H, s, H-19), 1.86 (3H, s, H-18), 1.08 (3H, s, H-15), 0.99 (3H, s, H-16), 0.96 (3H, s, H-20); NMR ^{13}C ($CDCl_3$, 100 MHz) δ_C 206.6 (C-10), 148.2 (C-17), 136.2 (C-9), 78.1 (CH-4), 76.2 (C-1), 58.4 (CH_2-11), 49.9 (CH-8), 49.1 (CH-14), 47.8 (CH_3-21), 42.9 (C-5), 39.7 (C-12), 38.6 (CH_2-6), 38.1 (CH_2-13), 34.4 (CH_2-2), 28.2 (CH_2-3), 27.4 (CH_2-7), 24.7 (CH_3-18), 22.9 (CH_3-19), 19.7 (CH_3-15), 19.2 (CH_2-20), 12.2 (CH_3-16). EIMS *m/z* 334 $[M]^+$ (14), 302 (2), 269 (1), 245 (2), 219 (2), 201 (2), 175 (3), 150 (16), 135 (8), 121 (7), 115 (7), 107 (11), 91 (12), 86 (100), 72 (11), 55 (18). Crystallographic data for compound **18**: $C_{21}H_{34}O_3$, formula weight (FW) = 334.5, temperature = 289 K, colorless needle, trigonal, space group *R*3, *a* = 28.2305(10) Å, *b* = 28.2305(10) Å, *c* = 12.6852(5) Å, *γ* = 120°, volume = 8755.2(7) $Å^3$, *Z* = 18, Dc = 1.14 g/cm^3, *μ* = 0.58 mm^{-1}, F(000) = 3312, crystal dimensions: 0.484 mm × 0.194 mm × 0.087 mm. Independent reflections: 6487 (Rint = 0.077). The final anisotropic full-matrix least-squares refinement on F^2 with 467 variables converged at R1 = 5.67%, for the observed data and wR2 = 15, 91% for all data. The Flack parameter was −0.1(2). CCDC No. 1957949.

(1S,4R,5R,8S,12R,14R)-4-Hydroxy-10-keto-1-methoxydolast-9-(17)ene (**19**).* White solid; $[\alpha]_D$ 43 (*c* 0.05, $CHCl_3$); ^1H NMR ($CDCl_3$, 500 MHz) δ_H 3.18 (1H, dd, *J* = 11.5, 4.2 Hz, H-4), 3.09 (3H, s, H-21), 3.00 (1H, dd, *J* = 8.5, 6.1 Hz, H-8), 2.38 (1H, m, H-3a), 2.25 (1H, d, *J* = 15.9 Hz, H-11a), 2.23 (1H, m, H-6a), 2.21 (3H, d, *J* = 2.5 Hz, H-19), 2.08 (1H, m, H-2a), 1.98 (1H, d, *J* = 15.9 Hz, H-11b), 1.88 (1H, d, *J* = 2.0 Hz, H-18), 1.82 (1H, m, H-13a), 1.78 (1H, m, H-13b), 1.61 (1H, m, H-3b), 1.38 (1H, m, H-6b), 1.20 (1H, m, H-2b), 1.20 (1H, m, H-14), 1.11 (3H, s, H-15), 1.08 (3H, s, H-16), 0.95 (3H, s, H-20); ^{13}C NMR ($CDCl_3$, 125 MHz) δ_C 206.7 (C-10), 148.2

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(C-17), 136.4 (C-9), 78.8 (CH-4), 76.4 (C-1), 58.7 (CH_2-11), 53.2 (CH-14), 49.4 (CH-8), 48.3 (CH_3-21), 43.2 (C-5), 39.8 (C-12), 38.4 (CH_2-13), 37.2 (CH_2-6), 32.8 (CH_2-2), 27.6 (CH_2-7), 26.8 (CH_2-3), 25.2 (CH_3-15), 24.7 (CH_3-18), 23.0 (CH_3-19), 19.6 (CH_3-20), 11.9 (CH_3-16); HRESIMS *m/z* 335.2605 $[M + H]^+$ (calcd for $C_{21}H_{35}O_3$, 335.2586)/

Compound ***20***. White solid; $[\alpha]_D$ −32 (*c* 0.05, $CHCl_3$); ^1H and ^{13}C NMR spectra were consistent with those reported by Fenical et al.[20]

Compound ***21***. It was identified in a mixture, and its ^1H and ^{13}C NMR spectra were consistent with those reported by Fenical et al.[20]

(4R,5R,8S,12R,14S)-4-Hydroxy-10-keto-dolasta-1(14),9(17)-diene (***22***). ^1H NMR ($CDCl_3$, 300 MHz) δ_H 3.63 (1H, dd, *J* = 10.4, 4.2 Hz, H-4), 2.19 (3H, d, *J* = 2.6 Hz, H-19), 1.90 (3H, d, *J* = 2.1 Hz, H-18), 1.61 (3H, s, H-15), 1.01 (3H, s, H-20), 0.73 (3H, s, H-16); ^{13}C NMR ($CDCl_3$, 75 MHz) δ_C 207.1 (C), 146.1 (C), 134.9 (C), 133.4 (C), 129.2 (C), 72.2 (CH), 58.9 (CH), 55.9 (CH_2), 43.0 (CH_2), 41.0 (C), 38.24 (CH_2), 38.19 (CH_2), 30.1 (C), 27.0 (CH_2), 23.7 (CH_3), 23.3 (CH_2), 23.23 (CH_3), 23.16 (CH_2), 21.1 (CH_3), 19.1 (CH_3); HRESIMS *m/z* 303.2322 $[M + H]^+$ (calcd for $C_{20}H_{31}O_2$, 303.2324).

Formation of Compound ***23*** *by an Acetylation of Compound* ***8***. Compound **8** (4.6 mg, 0.018 mmol) dissolved in triethylamine (1.0 mL) was treated with 100 μL of acetic anhydride and a catalytic quantity of 4-DMAP. The mixture was stirred at rt for 3 h. After that, 2.0 mL of H_2O was added, and the mixture was extracted with EtOAc (3 × 3.0 mL). The organic phases were washed with $NaHCO_3$- and NaCl-saturated solutions (3 × 3.0 mL) and dried with sodium sulfate. The residue obtained after the concentration was dissolved in CH_2Cl_2 and filtered through a silica gel pad to obtain compound **23** in quantitative yield.

(1R,3E,7Z,11S)-17-Acetoxy-13-ketodolabella-3,7,12(18)-triene (***23***). Colorless oil; $[\alpha]_D$ + 54 (*c* 0.06, $CHCl_3$); ^1H NMR ($CDCl_3$, 400 MHz) δ_H 5.28 (2H, m, H-3 and H-7), 4.68 (1H, d, *J* = 11.7 Hz, H-17a), 4.53 (1H, d, *J* = 11.7 Hz, H-17b), 2.74 (1H, d, *J* = 12.1 Hz, H-11), 2.41 (1H, m, H-6a), 2.38 (1H, d, *J* = 18.5 Hz, H-14a), 2.33 (1H, m, H-9), 2.28 (1H, m, H-5a), 2.21 (1H, d, *J* = 18.5 Hz, H-14b), 2.21 (3H, br s, H-20), 2.21 (1H, d, *J* = 18.5 Hz, H-14b), 2.20 (1H, m, H-5b), 2.11 (1H, m, H-2a), 2.06 (3H, s, H-22), 1.78 (3H, s, H-19), 1.68 (1H, m, H-10a), 1.61 (1H, m, H-2b), 1.43 (3H, s, H-16), 1.41 (1H, m, H-10b), 1.22 (3H, s, H-15); ^{13}C NMR (100 MHz, $CDCl_3$) δ_c 207.0 (C-13), 171.2 (C-21), 148.6 (C-18), 137.7 (C-12), 137.2 (CH-7), 135.5 (C-4), 131.0 (C-8), 125.2 (CH-3), 61.1 (CH_2-17), 54.7 (CH_2-14), 41.6 (CH-11), 41.0 (C-1), 40.0 (CH_2-2), 39.4 (CH_2-5), 34.5 (CH_2-9), 28.1 (CH_2-10), 24.6 (CH_3-19), 24.0 (CH_2-6), 23.1 (CH_3-15), 21.3 (CH_3-20), 21.0 (CH_3-22), 15.6 (CH_3-16); EIMS *m/z* 344 $[M]^+$ (6), 302 $[M- C_3H_6]^+$ (2), 284 $[M- CH_3CO_2H]^+$ (15), 241 (9), 201 (13), 173 (13), 161 (16), 150 (100), 135 (46), 121 (23), 107 (35), 91 (49), 79 (45), 67 (29), 55 (23).

Formation of Compound ***24*** *by an Acetylation of Compound* ***10***. Compound **10** (4.1 mg, 0.014 mmol) was treated with the same conditions used for compound **8** to obtain compound **24** in quantitative yield.

(1R,3E,7E,11S,13S)-13-Acetoxy-dolabella-3,7,12(18)-triene (***24***). White solid; $[\alpha]_D$ + 52 (*c* 0.03, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ_H 5.56 (1H, t, *J* = 6.5 Hz, H-13), 5.18 (1H, dd, *J* = 11.4, 4.4 Hz, H-3), 4.88 (1H, d, *J* = 10.5 Hz, H-7), 2.48, (1H, br d, *J* = 11.3 Hz, H-11), 2.03 (3H, s, H-22), 1.69 (3H, s, H-19), 1.65 (3H, s, H-17), 1.63 (3H, s, H-20), 1.43 (s, 3H, H-16), 1.16 (s, 3H, H-15); ^{13}C NMR (75 MHz, $CDCl_3$) δ_C 171.2 (C-21), 140.3 (C-18), 135.2 (C-4), 132.11 (C-8), 129.75 (CH-7), 125.5 (CH-3), 75.1 (CH-13), 48.9 (C-1), 46.4 (CH_2-2), 42.1 (CH-11), 40.7 (CH_2-5), 39.9 (CH_2-2), 38.2 (CH_2-9), 28.4 (CH_2-10), 24.3 (CH_2-6), 23.7 (CH_3-15), 22.1 (CH_3-20), 22.0 (CH_3-19), 21.5 (CH_3-21), 16.0 (CH_3-17), 15.4 (CH_3-16). EIMS *m/z* 270 $[M- CH_3CO_2H]^+$ (30); HRESIMS *m/z* 271.2501 $[M - AcOH]^+$ (calcd for $C_{20}H_{30}$, 271.2426).

Formation of Compound ***25*** *by an Acetylation of Compound* ***11***. Compound **11** (5.3 mg, 0.018 mmol) was treated with the same conditions used for compound **12** to obtain compound **25** in quantitative yield.

(1R,3E,7E,11S,13R)-13-Acetoxy-dolabella-3,7,12(18)-triene (***25***). Colorless oil; $[\alpha]_D^{25}$ 137 (*c* 0.03, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$) δ_H 5.59 (1H, d, *J* = 7.1 Hz, H-13), 5.22 (1H, dd, *J* = 11.2, 5.3 Hz, H-3), 4.89 (1H, d, *J* = 10.0 Hz, H-7), 2.60 (1H, d, *J* = 9.9 Hz, H-11), 2.47 (1H, m, H-2a), 2.35 (1H, m, H-6a), 2.26 (1H, m, H-5a), 2.24 (1H, m, H-9a), 2.16 (1H, m, H-5b), 2.12 (1H, m, H-6b), 2.06 (1H, m, H-9b), 2.01 (1H, m, H-14a), 1.57 (1H, m, H-14b), 1.52 (1H, m, H-2b), 1.44 (2H, m, H-10), 2.01 (3H, s, H-22), 1.69 (3H, s, H-20), 1.63 (3H, s, H-17), 1.60 (3H, s, H-19), 1.49 (3H, s, H-16), 1.09 (3H, s, H-15); ^{13}C NMR (75 MHz, $CDCl_3$) δ_C 171.1 (C-21), 141.6 (C-18), 135.2 (C-4), 132.0 (C-12), 132.0 (C-8), 129.6 (C-7), 125.7 (C-3), 74.9 (C-13), 47.8 (C-1), 47.7 (C-14), 42.5 (C-11), 39.9 (C-5), 39.2 (C-2), 38.1 (C-9), 28.0 (C-10), 24.3 (C-6), 23.2 (C-15), 22.0 (C-20), 21.8 (C-19), 21.3 (C-22), 16.2 (C-17), 15.5 (C-16). EIMS *m/z* 270 $[M- CH_3CO_2H]^+$ (30); HRESIMS *m/z* 271.2698 $[M - AcOH]^+$ (calcd for $C_{20}H_{30}$, 271.2426).

Formation of Compound ***26*** *by an Acetylation of Compound* ***8***. Compound **8** (4.7 mg, 0.018 mmol) was treated with the conditions used for compound **12** to obtain compound **25** in quantitative yield.

(1R,3Z,7R,8R,11S)-16-Acetoxy-13-ketodolabella-3,12(18)-diene (***26***). Colorless oil; $[\alpha]_D^{25}$ −10 (*c* 0.04, $CHCl_3$) ^1H NMR (400 MHz, $CDCl_3$) δ_H 5.70 (dd, *J* = 12.0, 5.2 Hz, 1H, H-3), 4.49 (d, *J* = 12.2 Hz, 1H, H-16a), 4.45 (d, *J* = 12.2 Hz, 1H, H-16b), 2.86 (d, *J* = 8.9 Hz, 1H, H-7), 2.56 (m, 1H, H-5a), 2.42 (d, *J* = 18.6 Hz, 1H, H-14a), 2.29 (m, 1H, H-5b), 2.26 (s, 3H, H-19), 2.24 (m, 1H, H-2a), 2.16 (d, *J* = 18.6 Hz, H-14b), 2.06 (s, 3H, H-22), 2.04 (m, 1H, H-9a), 1.97 (s, 3H, H-19), 1.90 (m, 1H, H-6a), 1.75 (m, 1H, H-2b), 1.69 (m, 2H, H6b and H10b), 1.37 (s, 3H, H-17), 1.36 (m, 1H, H-9b) 1.21 (s, 3H, H-15); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 205.9 (C-13), 171.1 (C-21), 150.3 (C-18), 137.0 (C-12), 135.0 (C-4), 131.0 (CH-3), 65.6 (CH-7), 60.6 (C-8), 60.4 (CH_2-16), 54.5 (CH_2-14), 42.2 (CH-11), 40.7 (C-1), 39.9 (CH_2-2), 36.9 (CH_2-9), 33.4 (CH_2-5), 27.6 (CH_2-10), 25.0 (CH_3-19), 23.6 (CH_2-15), 23.2 (CH_2-6), 21.9 (CH_3-20), 21.0 (CH_3-22), 17.8 (CH_3-17).

Cells and Viruses. African green monkey kidney cells (Vero) were grown in Dulbecco's Modified Eagle Medium (DMEM; Invitrogen, catalog (cat.) No. 11960) supplemented with 5% fetal bovine serum (FBS, Gibco), 2 mM L-glutamine (Invitrogen, cat. No. 25030). Antibiotics were added at final concentrations of 50 units/mL penicillin and 50 g/mL streptomycin (Invitrogen, cat. No. 15070). For the plaquing assay, carboxymethylcellulose (CMC) was added at 2%. ZIKV (ATCC VR-1839) and CHIKV strain RJ2016.0823, donated by Ferreira, Davis, department of Virology, UFRJ, were amplified in Vero cells.

Cytotoxicity Assay. The cytotoxic activity of the compounds on Vero cells was tested in vitro using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) method. In the 96-well plate previously treated with the compounds, the MTT reagent (Sigma-Aldrich) was added at a concentration of 5 mg/mL and incubated for 3 h at 37 °C. After this period, the MTT medium was removed, and 100 μM dimethyl sulfoxide (DMSO) was added to the plate by being incubated for 15 min to dissolve the formazan crystals.[23] The plate was subjected to an enzyme-linked immunosorbent assay (ELISA) reader at 550 nm absorbance. The percentage of metabolically active cells was compared to the control of untreated cells, determining the cytotoxicity of the compounds. These assays were performed three times independently, each in triplicate.

Antiviral Assay. To evaluate whether the compounds affect the replication of ZIKV and CHIKV, the cells were cultured in a DMEM medium and after confluence were infected with the ZIKV or CHIKV (multiplicity of infection (MOI) of 0.1) for 2 h at 37 °C and 5% CO_2 atmosphere. Then the viral inoculum was removed, and cells were incubated in the absence or presence of different concentrations of the compounds (1.25, 2.5, 5, 10, 15, 20, 25, and 50 μg/mL) for 4 d. The supernatant was harvested at day 4 and stored at −80 °C for a viral titer determination.

Titer Plaque Assay. To determine the viral titer, Vero cells were washed with phosphate-buffered solution (PBS), and a 2% (w/v) mixture of CMC (Sigma-Aldrich) was added with DMEM supplemented with 5% FBS, 5 mM L-glutamine, and 0.20% of sodium

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bicarbonate. Plates were evaluated daily and counted between 5 and 10 d of incubation at 37 °C with 5% CO_2. The viral titers were determined according to the number of viral plaque units per milliliter (PFU/mL) and EC_{50} value, which shows the concentration that inhibits 50% of the viral plaque formation. This was determined by a linear regression compared with an infected untreated control and infected treated with DMSO, to ensure that DMSO is not interfering with the inhibition of viral plaques. All determinations were performed three times independently, each in triplicate.

Virucidal Profile. The viral suspensions (ZIKV and CHIKV) were diluted in a DMEN medium in the presence or absence of the 5, 10, and 20 μM compounds and incubated at 37 °C for 4 h. The remaining virus titer obtained for each treatment was determined by a plaque assay as Vero cells, as described above.

Attachment Assay. Vero cells grown in 24-well plates were prechilled at 4 °C for 10 min and infected with ZIKV and CHIKV using an MOI of 0.1. The virus was adsorbed at 4 °C, in the presence or absence of various concentrations of compounds (5, 10, and 20 μM). After 2 h of adsorption at 4 °C, the unabsorbed virus was removed when the monolayer was washed with cold PBS, and then cells were covered with an overlaid medium. After 4 d, cells were fixed with 20% formaldehyde for 2 h and stained with crystal violet for 5 min. The plaques formed after each treatment were counted, and the titer was calculated.

Statistical Analysis. All assays were performed at least three times in triplicate, and a statistical analysis was made using the GraphPad Prism 4.0 program (GraphPad Software Inc.). The analysis of variance test was used, followed by multiple comparisons using the Kruskal–Wallis test. Differences were considered statiscally significant when $P < 0.05$.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.jnatprod.1c00199.

1D and 2D NMR spectra of all compounds and X-ray data for compound **18** (PDF)

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Notes

The authors declare no competing financial interest.

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