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Dolichocarpols A-F, Unprecedented Macrocyclic Humulene-Type Sesquiterpenoids from *Anaxagorea dolichocarpa*

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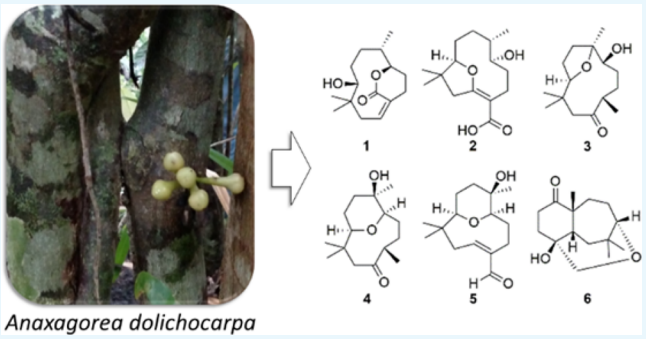
ABSTRACT: Six sesquiterpenoids with unprecedented macrocyclic humulene-type structures, namely, dolichocarpols A-E (**1**−**5**) with ether-bridged bicyclic rings between C-10 and C-2, C-10 and C-7, C-10 and C-6 (×2), and C-6 and C-3 and dolichocarpol F (**6**) cyclized between C-7 and C-2 and with an ether bridge between C-10 and C-3, were isolated from *Anaxagorea dolichocarpa* roots. The structures of the compounds were elucidated by NMR, MS, and IR data. Absolute configurations of compounds **1**−**3** and **6** were established on the basis of data from electronic circular dichroism, whereas relative configurations of compounds **4** and **5** were suggested by the NOESY spectrum. In addition, a putative biosynthetic pathway of the compounds is proposed. Furthermore, the cytotoxicity of **3**, **4**, and **6** against HCT-116 (human colorectal carcinoma) and L929 (murine fibroblast) was evaluated.



Anaxagorea dolichocarpa

INTRODUCTION

Sesquiterpenoids are considered to be the most diverse group of terpenoids containing a large variety of chemical structures that present different forms of derivation and cyclization from farnesyl diphosphate (FPP).[1,2] The humulene-type, an 11-membered macrocyclic sesquiterpenoid, is broadly found in plants, and previous reports have demonstrated various kinds of biological activities such as antitumor, anti-inflammatory, antibacterial, antiviral, and other bioactivities.[3−5]

The genus *Anaxagorea* (Annonaceae) comprises 30 species distributed in the neotropics and tropical Asia.[6] Previous phytochemical studies with plants of this genus revealed the presence of xanthones, flavonoids,[7−9] neolignans,[10] terpenoids, and alkaloids,[11−13] with some of them exhibiting remarkable biological activities. The analysis of the essential oil from the leaves of *Anaxagorea brevipes* has revealed the presence of mono- and sesquiterpenes such as α-, β-, and γ-eudesmol, guaiol, and caryophyllene oxide. In addition, its biological evaluation showed antimicrobial activity against Gram-positive bacteria and yeast and antiproliferative activity against breast, lung, and prostate cancer cell lines.[11] An interesting sesquiterpene called nordine besides copyrine alkaloids has been isolated from *A. javanica*, and some of these compounds have been assayed for nitric oxide (NO) inhibition.[13] *A. dolichocarpa*, widely distributed in South America,[14] is found in Brazil mainly in the North and Northeast regions.[15] Previous works exploring different parts of this plant reported the isolation of aporphine and azaphenanthrene alkaloids,[16,17] the identification of mono- and sesquiterpenes,[12,18] the phenolic quantification and determination of its antioxidant activity,[19] and a phytochemical screening and cytotoxicity evaluation of its extract.[20]

The gradual emergence of active agents derived from the plant kingdom into the fight against cancer has gained importance in the last decade.[21] These compounds have the potential to be harnessed for preventative strategies, standalone treatments, or complementary therapeutics in combination with traditional pharmacological interventions.[22] In addition, the literature has shown exciting data with respect to potential of natural compounds and their analogues in cancer prevention and treatment.[23]

In a continuing effort to isolate active compounds from plants in Brazil's semiarid area and motivated by the promising bioactivities and diversity of sesquiterpenoid structures, the

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ethanol extract of *A. dolichocarpa* roots was investigated. Thus, we describe here the isolation and structural elucidation of six humulene-type sesquiterpenoids (**1**−**6**). Furthermore, compounds **3**, **4**, and **6** were tested for cytotoxicity.

RESULTS AND DISCUSSION

The roots of *A. dolichocarpa* were dried and extracted with ethanol three times at room temperature. The ethanolic extract was suspended in MeOH-H_2O (7:3) and sequentially partitioned with *n*-hexane, chloroform, and ethyl acetate (EtOAc). The *n*-hexane- and chloroform-soluble phases were fractionated by silica gel chromatography column and medium-pressure liquid chromatography, respectively. The obtained fractions were then purified by preparative HPLC to yield the unprecedented macrocyclic humulene-type sesquiterpenoids dolichocarpols A (**1**) and E (**5**) from the hexane phase and dolichocarpols B-D (**1**−**4**) and F (**6**) from the chloroform phase. The structures were proposed after 1D- and 2D-NMR, IR, HRESIMS, ESIMS/MS, and experimental and calculated electronic circular dichroism (ECD) data analysis.

Compound **1** was isolated as a colorless oil. Its IR spectrum showed a hydroxyl group (3466 cm^{-1}) and a carbonyl group (1706 cm^{-1}). The molecular formula was determined as $C_{15}H_{24}O_3$ by HRESIMS based on the ion at *m*/*z* 275.1614 [M + Na]$^+$ (calcd for $C_{15}H_{24}O_3Na$, 275.1618, Δ = 1.2 ppm), corresponding to four indices of hydrogen deficiency. Of these, the ^{13}C NMR and DEPT spectra of **1** showed that one was associated with an olefinic group (δ_C 134.3, 133.2) and another with a carbonyl group (δ_C 168.8). Additionally, the spectrum showed signals assigned to two oxygenated carbons (δ_C 83.2, 82.3), one non-hydrogenated carbon (δ_C 39.3), one methine carbon (δ_C 37.7), five methylene carbons (δ_C 40.2, 31.7, 28.9, 27.3, 22.8), and three methyl carbons (δ_C 25.9, 22.0, 20.4) (Table 1). In the ^1H NMR spectrum, methyl groups could be inferred from two signals for tertiary methyl protons at δ_H 1.08 (s) and 0.82 (s) and one secondary methyl at 0.81 (d, *J* = 6.4 Hz), an olefinic proton at δ_H 5.84 (m), and two oxymethine proton at δ_H 4.58 (ddd, *J* = 9.2, 6.0, 2.0 Hz) and 3.10 (dl, *J* = 8.4 Hz) (Table 2). The comparison of these 1D NMR spectral data with those of nordine isolated from *A. javanica*[13] suggested that compound **1** was a macrocyclic humulene-type sesquiterpenoid as shown in Chart 1. In the HMBC spectrum, the correlations observed from H-10 to C-14, C-15, C-1, C-8, and C-9 and from H_2-1 to C-2 and C-3 localized a $\Delta^{2(3)}$ olefinic double bond (Figure 1). Moreover, correlations from H-6 to C-7, C-8, C-13, and C-12 and from H-2 and H_2-4 to C-3 and C-12 in combination with COSY correlations between H-8, H-7, and H-6 indicated an unusual ester fusion between C-6 and C-3. Also, in the COSY spectrum, the correlations between H_2-1, H-2 and H_2-4, H_2-5 established two pairs of allylic protons to C-2 and C-3. The ESIMS/MS spectrum of **1** exhibited ion fragments at *m*/*z* 257 [M - H_2O + Na]$^+$ and 217 [M - C_3H_6O + Na]$^+$. The relative configuration was deduced from NOESY experiments. The correlation between H-10, H_3-14, and H-2 indicated that these protons are cofacial adopting an α-orientation and assigning a β-orientation of OH-10 (Figure 2). In addition, the correlation from H-2 to H_3-14 suggested the *E*-configuration of the $\Delta^{2(3)}$ double bond, which is in agreement with an X-ray study that assigns the *E*-configuration to the $\Delta^{2(3)}$ double bond of the humulene skeleton.[24,25] The negative specific rotation ($[\alpha]_D^{25}$ −41, $CHCl_3$) when compared with other humulenes suggests the α-orientation to H_3-13 in this type of sesquiterpenoid, and the NOESY cross-peak from H_3-13 to H-6 revealed that they are cofacial with an α-orientation.[26] The ECD experimental spectra showed a negative Cotton effect at λ 234 nm (Δε −0.89) and a positive one at λ 255 nm (Δε +0.43); thus, the comparison of these with the calculated data allowed determination of the absolute configuration of **1** as (6*R*,7*S*,10*R*) (Figure 3). According to these lines of evidence, the structure of **1** was defined as humulene-type sesquiterpenoid named dolichocarpol A.

Table 1. ^{13}C (100 and 125 MHz) NMR Spectroscopic Data for Compounds 1−6 in $CDCl_3$

no	1	2[a]	3	4	5	6
1	40.2	45.3	47.1	55.4	39.4	33.6
2	134.3	173.0[b]	213.5	213.2	152.8	52.7
3	133.2	101.8[b]	47.0	49.4	147.8	74.1
4	28.9	22.2	30.6	32.2	18.1	33.0
5	22.8	33.6	37.5	26.4	26.3	32.9
6	82.3	67.5	75.5	84.6	83.5	215.9
7	37.7	31.7	84.0	69.4	69.9	50.5
8	31.7	32.2	27.7	32.8	34.3	29.9
9	27.3	26.1	29.8	20.9	20.6	23.7
10	83.2	92.3	85.6	73.4	72.7	82.6
11	39.3	40.0	39.6	39.9	36.1	39.5
12	168.8	170.6[b]	19.3	20.7	195.7	68.3
13	20.4	14.2	20.9	24.7	23.7	30.6
14	25.9	29.2	27.7	27.3	23.7	33.2
15	22.0	21.9	27.6	21.1	22.2	29.2

[a]^{13}C NMR (125 MHz). [b]^{13}C NMR (200 MHz).

Compound **2** was obtained as a colorless oil. Its IR spectrum showed a hydroxyl group (3401 cm^{-1}) and a carbonyl group (1671 cm^{-1}). The molecular formula was established as $C_{15}H_{24}O_4$ by HRESIMS *m*/*z* 291.1566 [M + Na]$^+$ (calcd for $C_{15}H_{24}O_4Na$, 291.1567, Δ = 0.1 ppm), indicating four indices of hydrogen deficiency. The ^1H NMR spectrum of **2** was similar to that of **1** except for the absence of an olefinic proton, thus still having three methyl groups and five methylene groups besides two oxymethine protons and one methine proton (Table 2). The ^{13}C NMR spectrum (125 MHz/$CDCl_3$) exhibited 12 carbon resonances, corresponding to two oxygenated carbons (δ_C 92.3, 67.5), one non-hydrogenated carbon (δ_C 40.0), one methine carbon (δ_C 31.7), five methylene carbons (δ_C 45.3, 33.6, 32.2, 26.1, 22.2), and three methyl carbons (δ_C 29.2, 21.9, 14.2) (Table 1). Other three non-hydrogenated carbons (δ_C 173.0, 170.6, 101.8) were detected in the 200 MHz ($CDCl_3$) ^{13}C NMR spectrum (Figure S21, Supporting Information). The HSQC spectrum revealed that the chemical shifts at δ_C 173.0, 101.8, and 170.6 were observed instead of those at δ_C 134.3, 133.2, and 168.8 assigned to C-2, C-3, and C-12 of compound **1**, respectively, and a double bond with the ether bridge between C-10 and C-2. In the ^1H and ^{13}C NMR spectra of **2** (Tables 1 and 2), the upfield chemical shifts observed for H-10 and C-10 and downfield chemical shifts of H-6 and C-6 supported the opening of the ester bridge C-6 and C-3 to form a C-6 hydroxyl. Similarly, the absence of H-2 and C-2 and downfield chemical shifts for C-2, C-3, and C-12 suggested an ether bridge between C-10 and C-2. In the HMBC spectrum, the correlation from H_2-1 to C-15, C-14, C-10, C-3, and C-2, together with the correlation from H-10 to C-2 and from H_2-4 to C-12, corroborated the connectivity between C-10 and C-2

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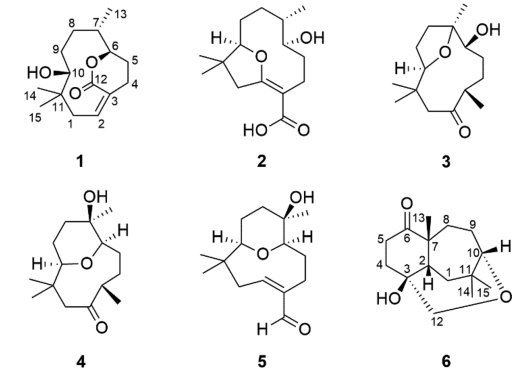
Table 2. ^{1}H (400 and 500 MHz) NMR Spectroscopic Data for Compounds 1−6 in $CDCl_3$

no	1	2[a]	3	4	5	6
1	2.88, td (13.6, 0.8)	2.97, d (19.5)	2.52, d (12.4)	2.34, dd (10.8)	3.12, t (11.2)	1.91, dd (16.0, 4.8)
	1.96, m	2.76, d (19.5)	1.84, d (12.4)	1.99, dd (10.8)	1.88, dd (11.2, 7.2)	1.66, dd (16.0, 2.8)
2	5.84, m				6.52, dd (10.8, 7.2)	2.29, m
3			2.74, m	2.71, dd (9.6, 6.8)		
4	2.51, m	2.39, m	1.89, m	1.86, m	2.61, dd (10.8, 1.2)	2.01, dt (13.2, 4.0)
	2.51, m	2.39, m	1.37, m	1.49, m	2.20, dd (13.2, 8.0)	1.74, ddd (13.6, 10.8, 5.6)
5	2.18, m	1.62, m	2.05, m	1.77, m	1.85, m	2.80, ddd (16.4, 13.2, 5.2)
	1.99, m	1.62, m	1.62, m	1.53, m	1.52, m	2.29, m
6	4.58, ddd (9.2, 6.0, 2.0)	3.57, dd (11.0, 4.0)	3.50, dd (5.2, 2.4)	3.52, m	3.58, dd (12.4, 5.6)	
7	2.22, m	2.56, m				
8	1.85, m	1.57, m	1.73, m	1.69, m	1.69, m	2.29, m
	1.34, m	1.57, m	1.62, m	1.48, m	1.56, m	1.45, ddd (14.8, 12.4, 7.6)
9	1.34, m	1.57, m	2.09, m	1.53, m	1.49, m	1.93−1.91, m
	1.19, m	1.45	1.21, m	1.40, m	1.30, m	
10	3.10, dl (8.4)	4.07, d (12.0)	3.48, dd (6.8, 4.0)	3.68, m	2.90, dd (11.2, 1.6)	3.52, dd (6.4, 2,4)
11						
12			0.96, d (7.2)	0.96, d (6.8)	9.37, d (0.8)	3.66, d (14.0)
						3.26, dd (14.0, 1.6)
13	0.81, d (6.4)	0.84, d (7.0)	1.09, s	0.99, s	0.98, s	1.09, s
14	1.08, s	1.09, s	1.24, s	0.91, s	0.84, s	1.23, s
15	0.82, s	1.07, s	0.85, s	1.03, s	1.00, s	1.05, s

[a] ^{1}H NMR (500 MHz).

Chart 1. Chemical Structures of Isolated Compounds 1−6 from Roots of *A. dolichocarpa*



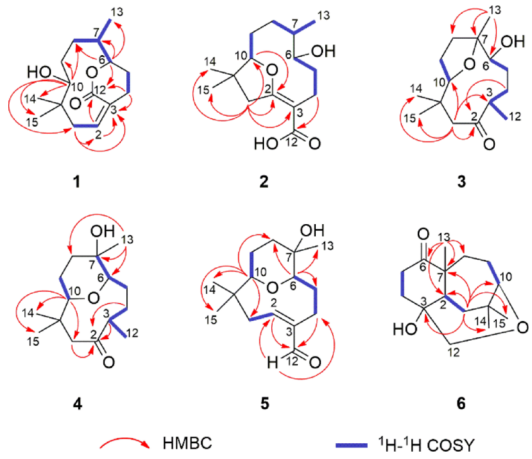


Figure 1. Key HMBC and ^{1}H-^{1}H COSY correlations of compounds 1−6.

(Figure 1). In the ^{1}H−^{1}H COSY spectrum of **2**, as expected, the correlation H-2/H_2-1 was not displayed, leaving those between H-10 and H_2-9, between H-8, H-7, and H_3-13, and between H-6, H_2-5, and H_2-4. The ESIMS/MS spectrum of **2** exhibited ion fragments at *m/z* 251 $[M - H_2O + H]^+$, *m/z* 233 $[M - 2H_2O + H]^+$, 215 $[M - 3H_2O + H]^+$, and 205 $[M - H_2O - HCOOH + H]^+$ compatible with a carboxyl group. The insertion of this group at C-3 is compatible with the chemical shift attributed to C-2. The NOESY correlation observed between H-10 and H_3-14 indicated an α-orientation to these protons (Figure 2). The multiplicity of the H-6 signal (dd) together with the absence of the ^{1}H−^{1}H COSY correlation with H-7 suggested that the dihedral angle between these protons is close to 90°. Thus, considering this evidence, the NOESY correlation between H-6 and H-7 indicated that these protons are cofacial with the β-orientation, so OH-6 and H_3-13 adopted an α-orientation, with a negative specific rotation ($[\alpha]_D^{25}$ −15, $CHCl_3$). The (6*S*,7*S*,10*R*) absolute configuration of **2** was determined by ECD experimental, with positive Cotton effects at λ 201 nm ($\Delta\varepsilon$ +1.55) and λ 243 nm ($\Delta\varepsilon$ +1.92), and calculated data (Figure 3). Therefore, the structure of compound **2**, named dolichocarpol B, was established as shown.

Compound **3** was also purified as a colorless oil. The IR spectrum showed a hydroxyl group (3458 cm^{-1}) and a carbonyl group (1703 cm^{-1}). The molecular formula of dolichocarpol C (**3**) was deduced as $C_{15}H_{26}O_3$ by HRESIMS at *m/z* 277.1773 $[M + Na]^+$ (calcd for $C_{15}H_{26}O_3Na$, 277.1774, Δ = 0.3 ppm), differing from compound **1** by the absence of the double bond. The 1D NMR data of compound **3** were similar to those of **1** and **2** but with an additional methyl carbon (δ_C 19.3) and a ketone carbonyl group (δ_C 213.5) instead of carbonyl ester or acid at C-3 (Tables 1 and 2). In the COSY spectrum, the combination of vicinal homonuclear coupling correlations observed between H-3, H_2-4, and H_3-12, between H-10 and H-9, and between H-6 and H_2-5 indicated an alteration in the junction of the carbon skeleton, compatible with a new bicyclic fusion, and defined the chemical shifts of the methine proton H-3 and oxymethine protons H-10 and H-

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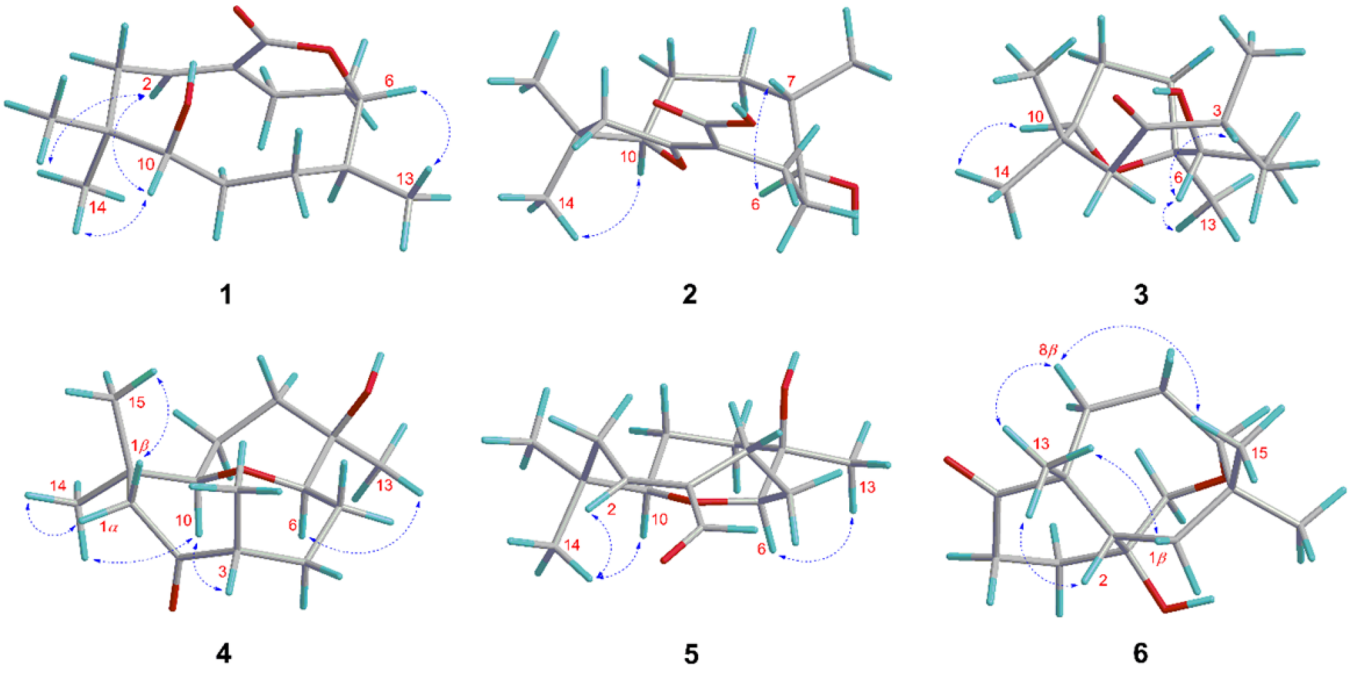

Figure 2. Key ^{1}H-^{1}H NOESY correlations of compounds **1**−**6**.

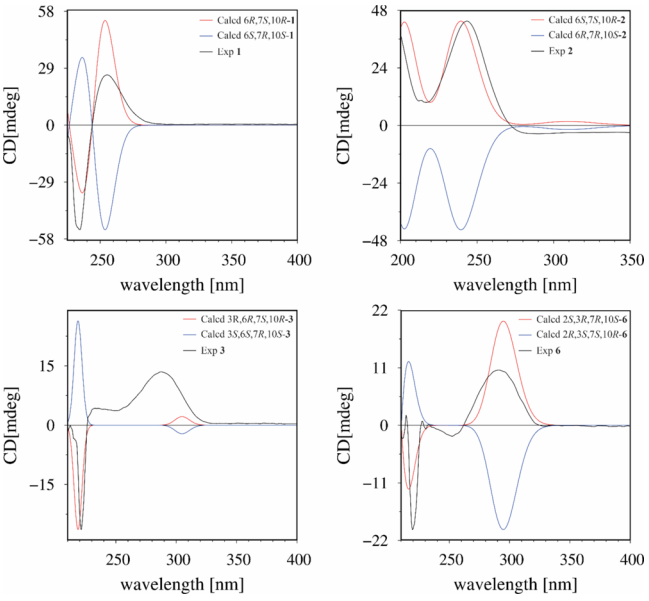

Figure 3. Experimental and calculated ECD spectra of compounds **1**−**3** and **6**.

6 (Figure 1). The HMBC cross-peak correlation from H_2-1 to C-15, C-14, C-10, and C-3 and from H_3-12, H_2-4, and H_2-1 to C-2 established that the ether bridge in **3** continued through C-10 but unlike the previous compounds. Additionally, the correlation from H_3-13 to C-8, C-7, and C-6 defined the ether bridge between C-10 and C-7. Compound **3** was acetylated, and only one acetate was obtained at C-6 (δ_H 4.61, dd, J = 5.2, 2.4, H-6). The HMBC spectrum of acetylated **3** (Figure S21, Supporting Information) showed correlations from H-6 to C-13, C-8, C-7, C-5, and C-4. This finding confirmed the bridge between C-10 and C-7, and the chemical shift at δ_C 84.0 was unequivocally assigned to C-7. The ESIMS/MS spectrum exhibited ion fragments at m/z 237 [M - H_2O + H]$^+$, 219 [M - $2H_2O$ + H]$^+$, and 201 [M - $3H_2O$ + H]$^+$. The analysis of specific rotation ([α]$_D^{25}$ −20, $CHCl_3$), ^{1}H NMR, and NOESY spectra of **3** recorded in both chloroform and pyridine defined the correlations between H-3, H-6, and H_3-13 and between H-10 and H_3-14, all with the same α-orientation, while H_3-12 and OH-6 adopted a β-orientation (Figure 2). The absolute configuration was established as (3*R*,6*R*,7*S*,10*R*) by comparing a negative Cotton effect at λ 221 nm ($\Delta\varepsilon$ −0.22) and a positive one at λ 287 nm ($\Delta\varepsilon$ +0.11) from experimental ECD with the calculated spectra. In view of the spectral data, including those of the acetylated derivative, the structure of **3** was determined as described and named dolichocarpol C.

Compound **4** was isolated as a colorless oil. Its IR spectrum showed a hydroxyl group (3461 cm^{-1}) and a carbonyl group (1705 cm^{-1}). The $C_{15}H_{26}O_3$ molecular formula was determined by HRESIMS data, m/z 277.1772 [M + Na]$^+$ (calcd for $C_{15}H_{26}O_3Na$, 277.1774, Δ = 0.6 ppm). The ^{1}H and ^{13}C NMR data of **4** (Tables 1 and 2) were similar to those of **3**, including a carbonyl (δ_C 213.2) and two oxymethine carbons (δ_C 73.4, 84.6). However, a significant alteration occurred at the quaternary carbinolic carbon C-7 (δ_C 69.4, Δ = +14.4 ppm). The HMBC spectrum displayed identical correlations from H_2-1, H-3, and H-4 to C-2, as in **3** (Figure 1). The cross-peaks from H-10 to C-15, C-14, and C-1 and from H_3-13 to C-8, C-7, and C-6 combined with vicinal homonuclear coupling correlations in COSY between H-10 and H_2-9, between H-7, H-6, and H_2-5, and between H-3, H_2-4, and H_3-12 suggested another type of fusion in the carbon skeleton of **4**. Analysis of the HSQC spectrum allowed an unequivocal establishment of the chemical shifts of C-10 (δ_C 73.4) and C-6 (δ_C 84.6). Thus, the changes in the chemical shifts were compatible with an ether bridge between C-10 and C-6 instead of C-10 and C-7 (Tables 1 and 2). As expected, compound **4** could not be acetylated. The ESIMS/MS spectrum of **4** exhibited ion fragments at m/z 237 [M - H_2O + H]$^+$, 219 [M - $2H_2O$ + H]$^+$, and 201 [M - $3H_2O$ + H]$^+$. The negative specific rotation ([α]$_D^{25}$ −12, $CHCl_3$) and the NOESY correlations observed from H-10 to H_3-14 and H-3 and from H-14 to H-1α suggest

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that these protons are cofacial in the α-orientation, whereas the correlations from H_3-15 to H-1β indicate a β-orientation (Figure 2). Furthermore, the correlation between H-6 and H_3-13 accompanied by previous data from the other compounds suggested the α-orientation for these protons and revealed the relative configuration shown in Figure 2. Thus, the structure of compound **4** was suggested as shown and named dolichocarpol D.

Compound **5** was obtained as a colorless oil. Its IR spectrum showed a hydroxyl group (3321 cm^{-1}) and a carbonyl group (1659 cm^{-1}). The 1D NMR data of compound **5** (Tables 1 and 2) were similar to those of compounds **1**, **2**, and **4**, altered by the presence of the formyl proton (δ_H 9.37, d, J = 0.8), as confirmed by HRESIMS at m/z 275.1622 $[M + Na]^+$ (calcd for $C_{15}H_{24}O_3Na$, 275.1618, Δ = −1.6 ppm), consistent with a molecular formula of $C_{15}H_{24}O_3$. The general characteristics observed in the ^1H and ^{13}C NMR spectra of **5** were parallel to those of the previous compounds. 2D NMR−HSQC analysis confirmed the chemical shifts for H-10 (δ_H 2.90, dd, J = 11.2, 1.6 Hz)/C-10 (δ_C 72.7), H-6 (δ_H 3.58, dd, J = 12.4, 5.6 Hz)/C-6 δ_C 83.5), and H-2 (δ_H 6.52, dd, J = 10.8, 7.2 Hz)/C-2 (δ_C 152.8). Thus, the ether bridge between C-10 and C-6 was maintained. The localization of the double bond in carbons C-2 and C-3 was defined by HMBC, through the identical sequence of the correlations from H-2 and H-4b to C-12 and from H-12 to C-2 and C-4, like in compound **1** (Figure 1). In addition, the cross-peaks from H-10 to C-15, C-14, C-8, and C-1 and from H-6 to C-13, C-8, and C-5 corroborated ether bridges C-10 and C-6. The $^1H-^1H$ COSY spectrum exhibited the vicinal coupling between H-2 and H_2-1, between H-10 and H-9, and between H-6, H-5, and H-4. The negative specific rotation ($[\alpha]_D^{25}$ −9, $CHCl_3$) and NOESY spectrum showed that the α-orientation was maintained for H-2, H-10, and H_3-14 as well as for H_3-13 and H-6, and the relative configuration is shown in Figure 2. From the analysis of the spectral data and comparison with the analogous compounds, the structure of **5** was proposed and named dolichocarpol E.

Compound **6** was isolated as a light brown oil. The IR spectrum showed a hydroxyl group (3444 cm^{-1}) and a carbonyl group (1701 cm^{-1}). Its molecular formula was determined as $C_{15}H_{24}O_3$ by HRESIMS analysis on the basis of ion m/z 275.1616 $[M + Na]^+$ (calcd for $C_{15}H_{24}O_3Na$, 275.1618, Δ = 0.8 ppm), corresponding to four indices of hydrogen deficiency. Unlike for compounds **1**−**5**, the ^1H NMR spectrum of **6** exhibited only one oxymethine proton at δ_H 3.52 (dd, J = 6.4, 2.4 Hz) and in addition two oxymethylene protons at δ_H 3.66 (d, J = 14.0 Hz) and 3.26 (dd, J = 14.0, 1.6 Hz). Besides these, there were one methine proton at δ_H 2.29 (m) and three methyl protons at δ_H 1.23 (s), δ_H 1.09 (s), and 1.05 (s) (Table 1). The ^{13}C NMR and DEPT spectra showed a carbonyl group (δ_C 215.9), three non-hydrogenated carbons including one oxygenated (δ_C 74.1, 50.5, 39.5), methylene carbons (δ_C 33.6, 33.0, 32.9, 29.9, 23.7), and methyl carbons (δ_C 33.2, 30.6, 29.2). The cross-peaks in HMBC from H_3-13 to C-8, C-7, C-6, and C-2 suggested a C-C fusion not found in other sesquiterpenoids (**1**−**5**) (Figure 1). Another correlation equally important from H_2-1 to C-15, C-14, C-10, C-7, C-3, and C-2 defined a new fusion between C-7 and C-2. The HSQC spectrum exhibited the correlation from H_3-13 to C-13, indicating its insertion in the quaternary carbon C-7 (δ_C 50.5), neighboring the carbonyl carbon. Likewise, the HMBC correlation from H_2-12 to C-10, C-3, and C-2, combined with the sequence of correlations of vicinal homonuclear coupling in COSY between H-10 and H_2-9, between H_2-1 and H-2, and between H_2-4 and H_2-5, established the ether bridge between C-10 and C-3 via methylene carbon C-12. The ESIMS/MS spectrum showed an ion fragment at m/z 215 $[M - C_2H_4O_2 + Na]^+$. The positive specific rotation ($[\alpha]_D^{25}$ +10, $CHCl_3$) indicates a different orientation for H_3-13 from those of the compounds shown previously. Furthermore, the NOESY correlation between H-2, H_3-13, H-8β, H_3-15, and H-1β suggested that all the protons were cofacial, thus assigned to the β-orientation (Figure 2). The Cotton effects observed at λ 219 nm ($\Delta\varepsilon$ −0.18) and at λ 291 nm ($\Delta\varepsilon$ +0.09) allowed establishment of the absolute configuration of **6** as (2*S*,3*R*,7*R*,10*S*) by the comparison of experimental ECD with the calculated data (Figure 3). On the basis of these findings, the structure of compound **6** was defined as a new humulene-type sesquiterpene, named dolichocarpol F.

A putative biosynthetic pathway toward compounds **1**−**6** is shown in Figure 4. Starting from 10-hydroxyhumula-2*E*,6*E*-diene, oxidations occurred producing the hydroxylated intermediate at C-10 and also the epoxidized intermediate at the double bonds $\Delta^{2,3}$ and $\Delta^{6,7}$. Through successive oxidations and different fusions and cyclizations, compounds **1**−**6** were produced.

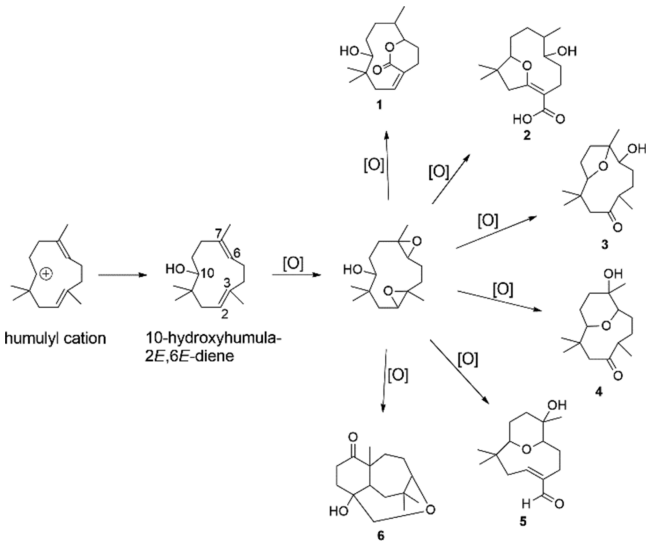

Figure 4. Putative biosynthetic pathway toward compounds **1**−**6**.

The cytotoxicity of the selected sesquiterpenoids (**3**, **4**, and **6**) against HCT-116 and L929 cell lines was evaluated. Compound **3** significantly inhibited the proliferation of HCT-116 human colon cancer cells at a concentration of 50 μM with an inhibitory rate of 26.63 ± 1.21% (Figure 5). No selectivity was observed for **3** toward tumor cells, considering the inhibitory rate against the L929 murine fibroblast nontumor cell line (27.91 ± 0.20%). Compounds **4** and **6** showed weak cytotoxic effects against HCT-116 cells with inhibitory rates under 20%. Moreover, compound **4** exhibited the highest cytotoxicity against L929 nontumor cells (30.76 ± 3.52%).

EXPERIMENTAL SECTION

General Experiment Procedures. Optical rotations were measured on a Jasco P-2000 polarimeter (Easton, MD, USA) at 25 ° C in $CHCl_3$. FTIR spectra were acquired on Bruker Vertex 70 (Bruker, Billerica, MA, USA) and PerkinElmer Frontier (Waltham, MA, USA) spectrometers. 1D- and 2D-

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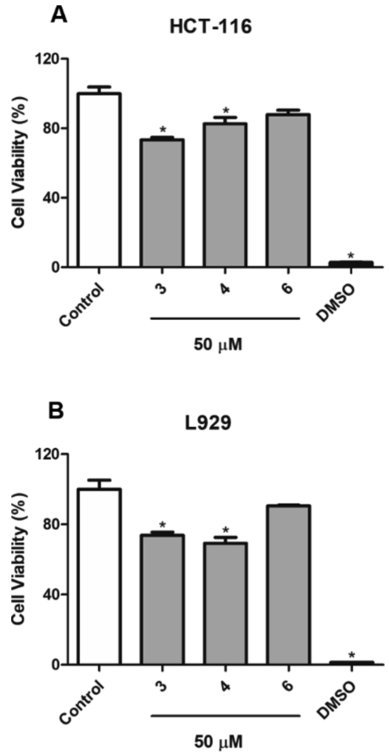

Figure 5. Cytotoxicity of compounds **3**, **4**, and **6** against HCT-116 (A) and L929 (B) cells lines, after 72 h of treatment. The values are presented as mean ± standard error for four independent replicates at a concentration of 50 μM (*p < 0.05 compared with untreated cells).

NMR spectra were recorded on a Bruker AVANCE III HD 800 MHz spectrometer (800 and 200 MHz for ^{1}H and ^{13}C, respectively), Varian NMR spectrometer (500 and 125 MHz for ^{1}H and ^{13}C, respectively), and Bruker AVANCE III HD spectrometer (400 and 100 MHz for ^{1}H and ^{13}C, respectively) using $CHCl_3$ (δ_H 7.24 and δ_C 77.0), the residual solvent, as an internal standard. High-resolution electrospray ionization mass spectrometry (HRESIMS) and tandem (MS^n) analyses were carried out using Bruker spectrometers of models micrOTOFII and Ion Trap-amaZonX (Billerica, MA, USA), respectively, both operating in the positive mode. Column chromatography (CC) was performed on a silica gel 60 (60−200 μm, 70−230 mesh, SiliaCycle, Quebec, Canada), and medium-pressure liquid chromatography (MPLC) was performed on a silica gel 60 (40−63 μm, 230−400 mesh, SiliCycle). Thin-layer chromatography (TLC) was carried out using precoated silica gel F-254 aluminum sheets (SiliCycle), and the compound spots were observed under UV light at 254 and 366 nm, staining with iodine vapor. Analytical high-performance liquid chromatography (HPLC) was performed on a Prominence Shimadzu instrument equipped with a SPD-M20A diode array detector and a reversed-phase Phenomenex Gemini C18 column (250 mm × 4.6 mm ID filled with 5 μm particles). For preparative HPLC, a Shimadzu apparatus with an SPD-M10A VP diode array detector and an ACE C18 column (250 mm × 21.2 mm and 5 μm particles) was used with a flow rate of 8.0 mL/min.

Plant Material. The roots of *A. dolichocarpa* were collected from Cruz do Espírito Santo, Paraíba, Brazil (7°09′43.7″S, 35°02′11.1″W) on April 2018. Access registration in the National Management System of Genetic Patrimony and Associated Traditional Knowledge (SISGEN) was obtained under number AE4B71A. A voucher specimen (AGRA & GÓES 5543) was identified by M. F. Agra and deposited at Herbarium Prof. Lauro Pires Xavier (JPB), Federal University of Paraiba (UFPB), Brazil.

Extraction and Isolation. The air-dried and powdered roots of *A. dolichocarpa* (1.72 kg) were extracted with ethanol for 72 h (3 × 4 L) at room temperature. The extract was concentrated under reduced pressure at 40 ° C to afford 52.6 g of crude extract (CE). Part of CE (49.3 g) was suspended in MeOH-H_2O (7:3) and sequentially partitioned with hexane, chloroform, and EtOAc. An aliquot of the hexane-soluble portion (8.12 g) was subjected to CC over silica gel, eluting with a gradient of hexane-EtOAc and EtOAc-MeOH, which after TLC analysis were grouped to give the fractions H-1 to H-6. Fraction H-2 (95.0 mg) was subjected to preparative HPLC with the following gradient elution: solvent A = H_2O; solvent B = MeOH; elution system = 0−40 min (20−70% B); 40−50 min (70% B); and 50−65 min (70−100% B), yielding compound **5** (2.0 mg). Fraction H-4 (85.8 mg) was also subjected to preparative HPLC using gradient elution with H_2O−MeOH 5 to 100% of MeOH in 75 min to afford compound **1** (1.0 mg). The chloroform-soluble fraction (6.70 g) was separated by MPLC over silica gel using a gradient elution with hexane−EtOAc and EtOAc−MeOH, and the fractions obtained were combined according to the TLC profile to afford C1−C4. The fraction C-2 (197.3 mg) was purified by preparative HPLC with the following elution gradient: solvent A = H_2O; solvent B = MeOH; elution system = 0−45 min (0−65% B); 45−70 min (65% B); and 70−105 min (65−100% B). This resulted in compounds **2** (0.5 mg), **3** (12.1 mg), **4** (6.8 mg), and **6** (6.0 mg).

Dolichocarpol A (**1**): colorless oil; $[\alpha]_D^{25}$ −41 (c 0.1, $CHCl_3$); ECD ($CHCl_3$) λ ($\Delta\varepsilon$) 234 (−0.89), 255 (+0.43); IR (film) ν_{max} 3466, 2955, 2931, 2868, 1706, 1468, 1402, 1213, 1104 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; positive-ion HRESIMS m/z 275.1614 $[M + Na]^+$ (calcd for $C_{15}H_{24}O_3Na$, 275.1618, Δ = 1.2 ppm); positive-ion ESIMS/MS fragments m/z 257, 217.

Dolichocarpol B (**2**): colorless oil; $[\alpha]_D^{25}$ −15 (c 0.1, $CHCl_3$); ECD ($CHCl_3$) λ ($\Delta\varepsilon$) 201 (+1.55), 243 (+1.92); IR (film) ν_{max} 3401, 2958, 2923, 2869, 1703, 1459, 1366, 1214, 1082, 1047 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; positive-ion HRESIMS m/z 291.1566 $[M + Na]^+$ (calcd for $C_{15}H_{24}O_4Na$, 291.1567, Δ = 0.1 ppm); positive-ion ESIMS/MS fragments m/z 251, 233, 215, 205.

Dolichocarpol C (**3**): colorless oil; $[\alpha]_D^{25}$ −20 (c 0.1, $CHCl_3$); ECD ($CHCl_3$) λ ($\Delta\varepsilon$) 221 (−0.22), 287 (+0.11); IR (film) ν_{max} 3458, 2956, 2924, 2873, 1703, 1458, 1367, 1215, 1048 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; positive-ion HRESIMS m/z 277.1773 $[M + Na]^+$ (calcd for $C_{15}H_{26}O_3Na$, 277.1774, Δ = 0.3 ppm); positive-ion ESIMS/MS fragments m/z 237, 219, 201.

Dolichocarpol D (**4**): colorless oil; $[\alpha]_D^{25}$ −12 (c 0.1, $CHCl_3$); IR (film) ν_{max} 3461, 2960, 2930, 2875, 1705, 1457, 1363, 1214, 1049 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; positive-ion HRESIMS m/z 277.1772 $[M + Na]^+$ (calcd for $C_{15}H_{26}O_3Na$, 277.1774, Δ = 0.6 ppm); positive-ion ESIMS/MS fragments m/z 237, 219, 201.

Dolichocarpol E (**5**): colorless oil; $[\alpha]_D^{25}$ −9 (c 0.1, $CHCl_3$); IR (film) ν_{max} 3321, 2975, 2929, 2883, 1659, 1453, 1383, 1270, 1082, 1045 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; positive-ion HRESIMS m/z 275.1622 $[M + Na]^+$ (calcd for $C_{15}H_{24}O_3Na$, 275.1618, Δ = −1.6 ppm).

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Dolichocarpol F (**6**): colorless oil; $[\alpha]_D^{25}$ +10 (*c* 0.1, $CHCl_3$); ECD ($CHCl_3$) λ ($\Delta\varepsilon$) 219 (−0.18), 291 (+0.09); IR (film) ν_{max} 3344, 2954, 2923, 2867, 1701, 1462, 1368, 1213, 1092 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; positive-ion HRESIMS *m*/*z* 275.1616 $[M + Na]^+$ (calcd for $C_{15}H_{24}O_3Na$, 275.1618, Δ = 0.8 ppm); positive-ion ESIMS/MS fragments *m*/*z* 215.

ECD Calculation. Conformational studies for compounds **1**−**6** were carried out on the Spartan'16 software using the MMFF94 molecular mechanics force field calculation. Conformers within a 10 kcal/mol energy window were generated and further optimized using density functional theory (DFT) calculation at the B3LYP/6-31G*(d) level. The conformers with over 1% of Boltzmann distribution were chosen for ECD calculation at the B3LYP/6-311+G(2d,p) level. The calculated ECD spectra were obtained by DFT and time-dependent DFT (TD-DFT) using Gaussian 09 and analyzed on SpecDis v1.71.

Cytotoxicity Assay. Human colon cancer HCT-116 cells and nontumor murine fibroblast L929 cells were cultured in an RPMI-1640 medium containing 10% fetal bovine serum, 100 U/mL penicillin, and 100 μg/mL streptomycin at 37 °C with 5% CO_2 in a humidified atmosphere. Cells were seeded in 96 well plates at a density of 3×10^5 cells per well. Following a 24 h period, cells were incubated with the sesquiterpenoids (**3**, **4**, and **6**) (50 μM) dissolved in DMSO (0.4%). After culturing for 72 h, 10 μL of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) solution (5 mg/mL) was added and incubated for another 4 h. The deposited formazan was dissolved with dodecyl sulfate sodium salt (100 μL).[27] Positive control was DMSO (20%). The optical densities were measured using a microplate spectrophotometer (Microplate reader BioTek Instruments, Sinergy HT, Winooski, VT, USA). Data were analyzed with GraphPad Prism 5.0 using the analysis of variance (one-way ANOVA).

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.0c00690.

HRESIMS, IR, NMR, and ESIMS/MS data of compounds **1**−**6** (PDF)

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Notes

The authors declare no competing financial interest.

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