Notes

24-Norhopene Derivatives from *Diatenopteryx sorbifolia*

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Two new hopene derivatives, 3*β*,6*β*-dihydroxy-21α*H*-24-norhopa-4(23),22(29)-diene (**1**) and 3*β*,5*β*-dihydroxy-6*β*-[(4-hydroxybenzoyl)oxy]-21α*H*-24-norhopa-4(23),22(29)-diene (**2**), together with cleomiscosin B (**3**) and 5,6-dimethoxy-7-hydroxycoumarin (umckalin), were isolated from the timber of *Diatenopteryx sorbifolia*. This is the first isolation of the norhopene skeleton from nature. The structures of the isolates were established by spectroscopic analysis.

The family Sapindaceae embraces 144 genera with 1325 species of tropical and subtropical trees, shrubs, lianas, and herbaceous climbers.[1] *Diatenopteryx* is represented by only two species. One of them, *Diatenopteryx sorbifolia* Radlk. (Sapindaceae), is a tree occurring in the Brazilian semi-arid region and is popularly known as "tingui". There are no previous phytochemical reports on the genus, and this work was initiated to investigate the cytotoxic constituents present in the $CHCl_3$-soluble fraction of the MeOH extract (LNCaP, ED_{50} 12.1 μg/mL).[2] Sequential chromatography on Si gel and Sephadex LH-20 afforded two triterpenoids possessing a new norhopene skeleton.

Compounds **1** and **2** revealed an intense blue color with the vanillin−H_2SO_4 reagent, and their ^{1}H- and ^{13}C-NMR spectra demonstrated their triterpene nature. The HREIMS of **1** exhibited a molecular ion peak at *m/z* 426 (obsd 426.3498; calcd 426.3501) indicating a molecular formula of $C_{29}H_{46}O_2$. The ^{1}H-NMR spectrum of **1** showed the characteristic signals of two oxy methine protons (*δ* 3.99; *δ* 4.46), one isopropenyl (*δ* 1.70; *δ* 4.70; *δ* 4.72), one terminal vinyl (*δ* 5.29; *δ* 5.30), and only four methyl groups. The ^{13}C-NMR spectrum displayed 29 peaks and confirmed the above data through the resonances displayed at *δ* 70.2, 73.2; *δ* 19.7, 109.6, 148.1; *δ* 104.5, 150.9; *δ* 15.0, 16.3, 16.9, 17.8, respectively. Analysis of the DEPT spectra also indicated the presence of four quaternary carbons and ten methylene and five methine groups. The hopane nature of **1** was indicated by the ^{13}C-NMR data, particularly, the resonances of carbons C-17, C-18 and the isopropenyl group in comparison with 21α*H*-hop-22(29)-ene,[3] hopene,[4,5] and lupene[5,6] derivatives. By comparing the chemical shifts, especially of C-19 and C-21, with those of 3*β*-methoxy hop-22(29)-ene[5] and 21α*H*-hop-22(29)-ene[3] (Table 1) it was possible to establish this skeleton for **1**. The ^{13}C-NMR spectrum also showed an oxy methine signal at *δ* 73.2 that was attributed to C-3. This resonance was upfield when compared with other triterpenes bearing a hydroxyl group at C-3,[3−5] suggesting the absence of a methyl group at C-4. In support of this, the DQCOSY spectrum indicated correlations between the vinylic protons (*δ* 5.29) and the oxymethine proton H-3 (*δ* 3.99). This evidence indicated that the vinyl group was attached at C-4 and suggested a novel 21α*H*-24-norhopa-4(23),22(29)-diene skeleton for **1**.

Table 1. ^{1}H-NMR Spectral Data for **1** and **2** ($CDCl_3$)

proton	**1**	**1a**	**2**
2*β*	1.60[a]		
2α	1.90[a]		
3α	3.99 (dd; 11.0, 5.57)	5.09 (dd; 12.21, 5.16)	3.95 (m)[a]
5α	1.70 (m)[a]		1.75 (m)[a]
6α	4.46 (d, 1.0)	4.42 (d; 1.0)	4.43 (d; 1.65)
7α	1.50[a]		5.27 (d; 1.68)
7*β*	1.60		
9	1.40[a]		1.30[a]
13	1.50[a]		1.60[a]
15*β*	1.19[a]		
17	1.00 (m)[a]		1.00 (m)[a]
20*β*	1.10		
20α	2.00[a]		
21α	2.30 (m)[a]		2.23 (m)[a]
23	5.29, 5.30 (br s)	4.99, 5.24 (br s)	5.20, 5.36 (br s)
25	1.03 (s)	1.01 (s)	1.06 (s)
26	1.42 (s)	1.37 (s)	1.62 (s)
27	0.95 (s)	0.90 (s)	1.07 (s)
28	0.71 (s)	0.70 (s)	0.68 (s)
29	4.70, 4.72 (br s)	4.66, 4.68 (br s)	4.64 (dd; 1.23, 6.24)
30	1.70 (br s)	1.65 (br s)	1.65 (s)
2′, 6′			7.96 (d; 8.64)
3′, 5′			6.89 (d; 8.67)
H_3CCO		2.12 (s)	

[a] Chemical shifts were assigned from DQCOSY and HMQC.

The correlations observed in the HMBC spectrum fixed the spatial relationships among the methyl, vinyl, and isopropenyl groups mentioned above. Thus, correlations between C-9 (*δ* 48.7) with Me-25 (*δ* 1.03) and Me-26 (*δ* 1.42), as well as between C-13 (*δ* 47.5) with Me-27 (*δ* 0.95) and Me-28 (*δ* 0.71), confirmed the A/B/C/D ring linkages. Correlations found for Me-25 fixed, unambiguously, the chemical shift for C-5, and, through the correlations observed between C-5 and the H_2-vinylic with H-3, it was demonstrated that the A ring possessed an *exo*-methylene group.

The EIMS base ion at *m/z* 189 indicated an unsubstituted D/E ring[7] for **1**. Moreover, the location of a hydroxyl group at C-6 was suggested from the ^{13}C-NMR spectrum. When **1** was compared with literature data both C-6 (Δ*δ* +51.4 ppm) and C-7 (Δ*δ* +5.6 ppm) were deshielded.[3−5] HMBC spectroscopy supported the above observation with a correlation displayed between Me-

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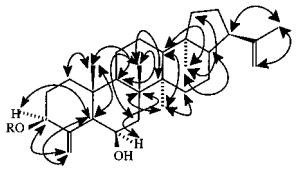


Figure 1. HMBC data of **1**.

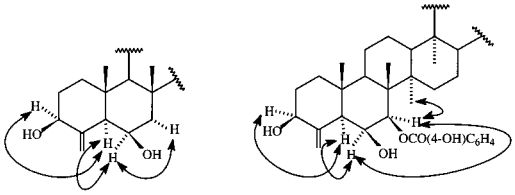


Figure 2. Significant NOESY interactions of **1** and **2**.

25 (δ 1.42) and C-7 (δ 39.0). Figure 1 summarizes the correlations observed in the HMBC spectrum of **1**.

The DQCOSY spectrum, besides the correlations previously mentioned, showed correlations between H-5 and the oxy methine proton at δ 4.46 (H-6), and for H-6 with H-7 (δ 1.55). This evidence confirmed the spatial disposition of the groups present in the A and B rings of **1**. The coupling constant recorded for H-6 (d, J = 1.0 Hz) in the ^{1}H-NMR spectrum indicated an equatorial position for this proton. NOESY experiments (Figure 2) corroborated the early observation and established a β-orientation for the hydroxyl group attached at C-6 through correlations displayed between δ 4.46 (H-6) with the resonances at δ 1.70 (H-5*ax*) and δ 1.50 (H-7*ax*).

Acetylation of **1** produced only a 3-monoacetylated derivative **1a**, suggesting steric hindrance for the C-6 hydroxyl group. The molecular formula $C_{31}H_{48}O_3$ of **1a** was indicated by the ^{13}C-NMR data, as well as by HREIMS (obsd 468.3592, calcd 468.3603). The ^{1}H-NMR spectrum displayed signals for methine protons at δ 5.09 and δ 4.43. The former peak showed strong ^{1}H$-^1$H COSY correlations with both H-23 (δ 4.99 and 5.24) and H_2-2, demonstrating that acetylation had occurred at C-3. On the other hand, H-23 showed correlations with H-3 and H-5 (δ 1.70). The ^{13}C-NMR data (Table 2) agreed with these observations with the anticipated shifts occurring for C-2, C-4, and C-23.

HREIMS (obsd 562.3660) and ^{13}C-NMR data established the molecular formula $C_{36}H_{50}O_5$ for the isolate **2**. Like **1**, compound **2** was a 24-*nor*-21α*H*-hopa-4(23), 22(29)diene, which differed from **1** by the presence of an additional (4-hydroxybenzoyl)oxy group. The former inference was based on the chemical shifts of C-3, Me-26, and the vinyl and isopropenyl groups observed in the ^{13}C NMR, and by correlations obtained in the DQCOSY spectrum. The presence of a (4-hydroxybenzoyl)oxy group was demonstrated through ^{1}H- (Table 1) and ^{13}C-NMR (Table 2) spectra, showing doublets at δ 6.8 and 7.96 and resonances at δ 116.0, 122.0, 132.5, 160.0, and 165.0, respectively. EIMS confirmed these observations through the presence of a base peak at m/z 121.

DQCOSY and HMBC (Figure 3) experiments established the relationships between the hydroxyl and (4-hydroxybenzoyl)oxy groups. The HMBC spectrum showed a correlation between the methine signal at δ 5.27 and the resonance at δ 13.2 (CH_3-26), which indicated the presence of a (4-hydroxybenzoyl)oxy group

Table 2. ^{13}C-NMR Spectral Data for **1** and **2** ($CDCl_3$)

carbon	**1**[a,b]	**1a**[a]	**2**[a,c]	21α*H*-hopene[3]	3β-methoxy-hop-22(29)-ene[5]
1	40.1	39.9	40.9	40.4	38.9
2	32.1	28.5	32.9	18.6	22.4
3	73.1	74.5	73.7	42.2	88.9
4	150.9	145.8	147.7	33.3	39.0
5	52.2	52.3	50.2	56.2	55.9
6	70.2	70.0	72.2	18.8	18.5
7	39.0	39.1	75.9	33.4	33.9
8	41.3	41.3	46.4	42.3	42.0
9	48.7	48.7	49.8	50.5	50.6
10	38.3	38.2	38.1	37.5	37.4
11	20.9	20.9	21.1	21.0	21.3
12	23.9	23.9	24.6	24.1	24.2
13	47.5	47.5	48.6	48.8	49.7
14	42.7	42.7	43.9	42.0	42.3
15	32.5	32.5	35.6	32.7	33.6
16	21.5	21.5	21.3	21.0	21.9
17	53.9	53.9	54.1	54.0	55.1
18	44.2	44.2	44.0	44.3	45.0
19	40.2	40.2	41.1	40.3	42.1
20	27.3	27.3	27.6	27.4	27.6
21	47.9	47.9	48.4	48.0	46.7
22	148.1	148.1	148.8	148.3	148.8
23	104.5	105.4	105.9	33.5	28.3
24				21.7	16.3
25	16.3	16.2	16.6	16.0	16.3
26	17.8	17.8	13.2	16.7	16.7
27	16.9	16.8	17.9	16.9	16.7
28	15.0	15.0	15.7	15.2	16.0
29	109.6	109.6	110.2	109.5	110.3
30	19.7	19.7	20.3	19.7	25.2
1′			122.0		
2′, 6′			132.5		
3′, 5′			116.0		
4′			160.0		
7′			165.0		

[a] Multiplicity in ^{13}C obtained by APT, DEPT 135°, and DEPT 90° experiments. [b] Chemical shifts assigned from HETCOR and HMBC. [c] Chemical shifts were assigned from HMBC.

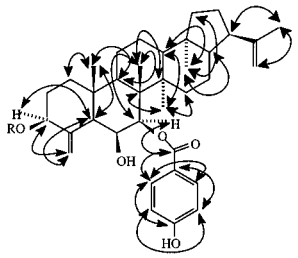
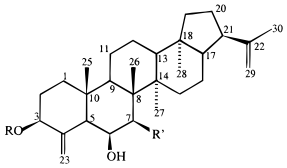


Figure 3. HMBC data of **2**.

at C-7. In addition, the DQCOSY spectrum displayed correlations between H-3 (δ 3.95) and H-5 (δ 1.75) and H-6 (δ 4.43) and H-7 (δ 5.27), establishing the presence of vicinal methine protons. The ^{13}C-NMR spectra of **2** when compared with those of **1** corroborated the evidence previously reported, showing the presence of an additional oxy methine carbon at δ 75.9 and the absence of the absorption at δ 39.0. A shielding effect was noted for C-5 ($\Delta\delta$ −2.0 ppm) for the ester moiety in a γ position and a deshielding effect for C-8 ($\Delta\delta$ +5.0 ppm) and C-6 ($\Delta\delta$ +2.2 ppm).

The 6β-hydroxy and 7β-(*p*-hydroxybenzoy)loxy orientations were indicated by the ^{1}H-NMR and NOESY experiments. The coupling constants observed for the H-6 (J = 1.65) and the H-7 (J = 1.68) were in agreement with the NOESY experiment (Figure 2) in which were displayed correlations between the resonance at δ 4.43 (H-6) and the signal at δ 5.27 (H-7), and for the latter with the resonance at δ 1.07 (Me-27).

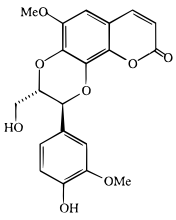
Compound **3** was identified as cleomiscosin B by comparison of physical and spectroscopic data,[8,9] and 5,6-dimethoxy-7-hydroxycoumarin (umckalin) was similarly identified.[10] Cleomiscosin B was reported to possess weak activity in the P-388 lymphocytic leukemia test system.[11]



1: R = H; R' = H

1a: R = $COCH_3$; R' = H

2: R = H; R' = OCO(4-OH) C_6H_4



3

Experimental Section

General Experimental Procedures. TMS or $CDCl_3$ was used as internal standard ($\delta_{TMS} = 0$ ppm; $\delta_{CDCl_3} = 7.27$ ppm). Chemical shifts are reported in δ (ppm), and the coupling constants (J) are measured in Hz. NOESY, ^{1}H- and ^{13}C-NMR experiments were obtained on a Varian XL-300 spectrometer (^{1}H, NOESY, DQCOSY, ^{13}C, and APT). DEPT 135° and DEPT 90° were performed on a Nicolet NT-360 (360 MHz) spectrometer operating at 90.8 MHz. HMQC and HMBC spectra were recorded on a GE Omega 500 MHz instrument (499.9 MHz for ^{1}H NMR) with the standard programs. Column chromatography was carried out on Si gel 60 (70−230 mesh, Merck). TLC was conducted over precoated sheets and plates of Si gel 60 F_{254}.

Plant Material. Leaves and trunk wood of *Diatenopteryx sorbifolia* Radlk. were collected by Professor Wilson A. Lopes near Valente−BA (Brazil) in March 1996. The plant material was identified by Dr. Marcelo Fonseca, and a voucher specimen was deposited at the Herbarium Alexandre Leal Costa of the Universidade Federal da Bahia (HALC/IB/UFBA) under number 024250.

Extraction and Isolation. The powdered wood (2.8 kg) was repeatedly extracted with MeOH at room temperature. The concentrated extracts were partitioned successively between hexane−MeOH−H_2O (9:1) and $CHCl_3$−MeOH−H_2O (6:4). The $CHCl_3$ phase (8.6 g) was filtered over a Si gel column using mixtures of $CHCl_3$−EtOAc of increasing polarity. The residue (2.15 g) afforded from the $CHCl_3$−EtOAc (9:1) filtrate was column chromatographed on Si gel eluting with petroleum ether−EtOAc. The fractions eluted with petroleum ether−EtOAc 6:4 (101.6 mg), 1:1 (258.2 mg), and 3:7 (130.1 mg) and with EtOAc (317.2 mg) were further purified. Thus, **1** (33.2 mg, 1.18×10^{-3} %) was obtained by column chromatography on Si gel with $CHCl_3$−EtOAc (7:3), and **2** (15.8 mg, 5.6×10^{-4} %) resulted from column chromatography on Si gel followed by preparative TLC, both using $CHCl_3$−EtOAc (1:1). Compound **3** (3.7 mg, 1.2×10^{-4} %) was isolated from a fraction eluted with petroleum ether/EtOAc (3:7) by column chromatography on Si gel eluting with $CHCl_3$−EtOAc (6:4). The fraction obtained with pure EtOAc afforded 5,6-dimethoxy-7-hydroxycoumarin (umckalin, 4.0 mg, 1.4×10^{-4} %) by further purification over column chromatography on Si gel eluting with $CHCl_3$−EtOAc (6:4).

3*β*,6*β*-Dihydroxy-21α*H*-24-norhopa-4(23),22(29)-diene (1): oil; $[\alpha]_D$ +13° (*c* 0.45, $CHCl_3$); IR ($CHCl_3$) ν_{max} 3460 (OH), 2953, 2854, 1657, 1592, 1464, 1368, 1208, 1080, and 920 cm^{-1}; ^{1}H NMR (see Table 1); ^{13}C NMR (see Table 2); HREIMS *m*/*z* 426.3498 (calcd for $C_{29}H_{46}O_2$, 426.3501); EIMS *m*/*z* 426 [M$^+$] (61), 411 (6), 393 (4), 189 (100).

Acetylation of 1. Addition of Ac_2O (0.5 mL) to **1** (5.6 mg) in pyridine (0.5 mL) at room temperature for 12 h, followed by purification of the acetylated compound in the usual way afforded **1a**; oil; IR ($CHCl_3$) ν_{max} 3507 (OH), 2942, 2872, 1743 (C=O), 1636, 1419, 1395, 1238, 1034, 768 cm^{-1}; ^{1}H NMR (see Table 1); ^{13}C NMR (see Table 2); HREIMS *m*/*z* 468.3592 (calcd for $C_{31}H_{48}O_3$, 468.3603); EIMS *m*/*z* 468 [M$^+$] (3), 408 (16), 393 (4), 189 (100), 107 (60).

3*β*,5*β*-Dihydroxy-6*β*-[(4-hydroxybenzoyl)oxy]-21α*H*-24-norhopa-4(23),22(29)-diene (2): oil; $[\alpha]_D$ +10° (*c* 0.79, $CHCl_3$); IR ($CHCl_3$) ν_{max} 3435 (OH), 2930, 2874, 1712 (C=O), 1593, 1464, 1368, 1280, 1168, and 760 cm^{-1}; ^{1}H NMR (see Table 1); ^{13}C NMR (see Table 2); HREIMS *m*/*z* 562.3658 (calcd for $C_{36}H_{50}O_5$, 562.3660); EIMS *m*/*z* 562 [M$^+$] (9), 547(2), 424 (7), 202 (15), 189 (24), 121 (100).

Biological Activity. None of the pure isolated compounds showed activity when tested in a panel of human cancer cell lines.[2]

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